North Carolina Beach Monitoring Program Quality Assurance Project Plan

June 16, 2003 Revised April 1, 2021



North Carolina Department of Environmental Quality
Division of Marine Fisheries

Shellfish Sanitation & Recreational Water Quality Section

Mission

To protect the public health by monitoring the quality of North Carolina's coastal recreational waters and notifying the public when bacteriological standards for safe bodily contact are exceeded.

Prepared by:

Erin Bryan-Millush Project Manager, Recreational Water Quality Program

Approving Signatures:

Kathy B. Rawls, Division of Marine Fisheries Shannon Jenkins, Section Chief Gary Bennett, QA Reviewer, EPA Joel Hansel, Project Manager, EPA

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SECTION A: PROJECT MANAGEMENT

A3 - Distribution List

Joel Hansel, EPA
John Batherson, Act. Director, DMF
Patricia Smith, PIO, DMF
NC Shellfish Sanitation & Recreational Water Quality (Wilmington)
NC Shellfish Sanitation & Recreational Water Quality (Nags Head)
Connie Brower, NC Division of Water Resources
Rachel T. Noble, Ph. D. Institute of Marine Sciences, UNC
Zack Moore, Division of Epidemiology
Coastal County Health Directors

A4 - Project / Task Organization

Shannon Jenkins, Section Chief, Shellfish Sanitation and Recreational Water Quality Section Morehead City Office

Andy Haines, Assistant Section Chief, Shellfish Sanitation and Recreational Water Quality Section Morehead City Office

Erin Bryan-Millush, Project Manager, Public Notification, Laboratory Quality Assurance Officer

Patricia Smith, Public Information Officer, DMF (Morehead City) Media Notification

The following staff members have responsibilities in both Shellfish and Recreational Programs:

Morehead City Office

Amanda Hewes, Laboratory Manager
Wayne Hall, RWQ Data Management and Lab Methods
Zach Fasking, Sample Collection, Data Entry, Laboratory Assistance
Phil Piner, Sample Collection, Laboratory Assistance
Timmy Moore, Sample Collection, Laboratory Assistance
Jason Hill, Sample Collection, Laboratory Assistance, Purchasing & Inventory
Andrew Haines, Sanitary Surveys

Wilmington Office

Holly White, Laboratory Manager Jeremy Humphrey, Shoreline Survey Matthew Stokely, Sample Collection, Data Entry, GPS Vacant, Shoreline Survey Lucas Edmondson, Sample Collection Nags Head

KC McAvoy, Laboratory Manager Kenny Midgett, Sample Collection, Laboratory, GPS Christa Sanderford, Shoreline Survey, Sample Collection, GPS

A5 - Problem Definition / Background

Coastal North Carolina has an abundance of surface water resources: 320 miles of Atlantic Ocean shoreline, 4,000 miles of estuarine shoreline and 2 million acres of shellfish growing waters. The beaches, coastal rivers and sounds play a large part in North Carolina's prosperous tourism industry, attracting more than 15 million vacationers each year. The resident population is growing rapidly, as more people find the North Carolina coast a desirable place to live and retire. As the population continues to grow, water quality is expected to decline as a result of increased run-off from land-disturbing activities.

In the mid-1990s, *pfiesteria* emerged in the national media as a possible public health threat, and North Carolina's Neuse River was in the spotlight for harboring the dinoflagellate organism. At that time, *pfiesteria* was found only in the brackish waters of the Neuse River, but the negative publicity carried the underlying possibility that all of North Carolina's coastal waters were unsafe for the consumption of seafood or for swimming. Water quality issues were rekindled in 1996 when a report from the Natural Resources Defense Council (NRDC) labeled North Carolina as a Beach Bum state for its lack of beach monitoring and public notification programs. When the legislature assembled the next year, it took very little lobbying to convince lawmakers to fund a recreational water quality program for coastal waters. In June 1997 the North Carolina Shellfish Sanitation Section was charged with monitoring coastal waters for two years as a pilot project. Approximately 300 sites were monitored weekly during the swimming season using fecal coliform as the indicator organism. As a result of the two years of monitoring, the reputation of North Carolina's coastal waters started improving, and public confidence in the health and safety of the coastal resources was being restored. In 1999 the program was funded permanently.

This document will report new program requirements and standards necessary for compliance with the Environmental Protection Agency s (EPA) guidance and will be a guide or protocol for operating the program. One aspect of the program that will not change is the protocol for posting swimming advisories and methods for notifying the public. Advisories will still be based on the exceedance of the bacteriological standard; however, the standard will be enterococci instead of fecal coliform. The posting of advisories for single-sample maximum exceedances is another significant change to the program.

A6 - Project/Task Description and Schedule

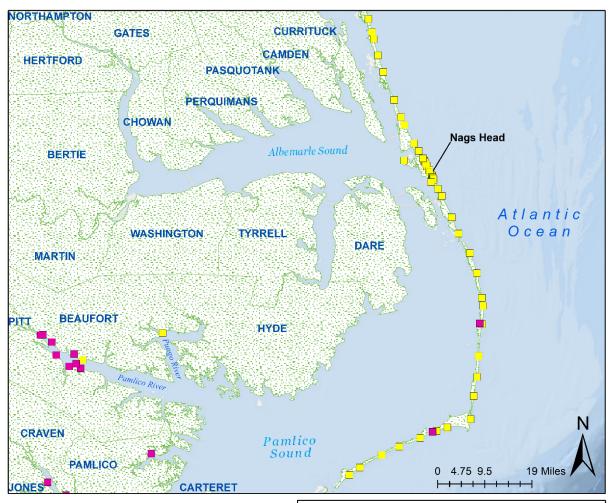
The coastal counties are divided into three regions: Northern, Central and Southern (see Figures 1, 2, 3). The three regions combined have approximately 213 sites that are monitored either weekly or twice monthly during the swimming season. Monitoring sites that are adjacent to resort areas and public accesses, that are used daily, are Tier I beaches and are sampled weekly. Monitoring sites located along the ICW, tidal creeks & summer camps, that are not used daily, are Tier 2 beaches and are sampled twice monthly.

The Shellfish Sanitation and Recreational Water Quality Section (SSRWQ) has a State and Food and Drug Administration (FDA)-accredited laboratory in Morehead City and Wilmington. A small laboratory at the NC Coastal Reserve Office in Kitty Hawk NC is setup just to process recreational water quality samples using IDEXX Enterolert. Sample collection, laboratory analysis, and beach monitoring activities are conducted entirely by the SSRWQ staff; however, Dare County Health Department in the northern region has an agreement with the SSRWQ to issue the public notification locally for the Dare County swimming advisories. The state Division of Marine Fisheries then follows up with public notification to the Associated Press.

The State will continue to fund the Tier II monitoring sites, approximately 50% of all the swimming areas, while the EPA grant will be used to fund the monitoring of the Tier I beaches. Appendices 1, 2 and 3 list the monitoring sites that are supported by the state of North Carolina and the EPA grant. Appendix 4 contains the coordinates for each monitoring site or swimming area.

The EPA grant is also used to fund public education and outreach. Appendix 8 contains educational materials used at the public meetings; they are required by the EPA to be grant-eligible.

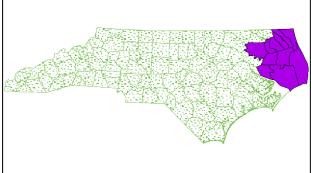
NC Recreation Water Quality Program
Northern Region



Tier Identification and Regional Locations

Monitoring Stations

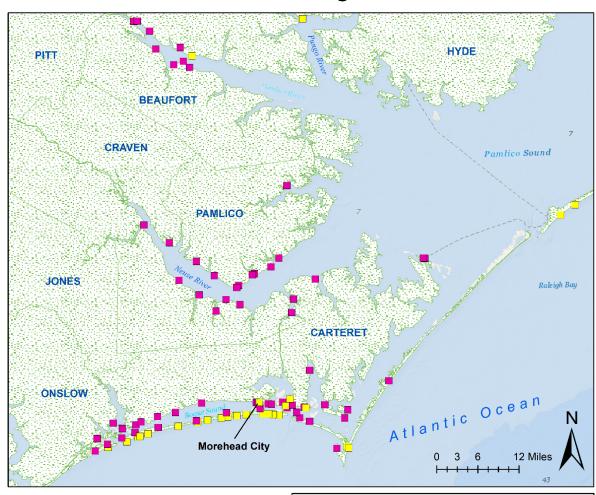
- 1 Daily Use
- 2 Non-daily Use





Shellfish Sanitation and
Recreational Water Quality
June 2021
Not to be used for navigational purposes.

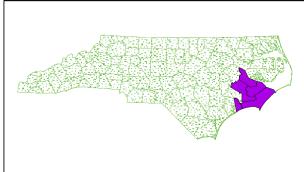
NC Recreation Water Quality Program
Central Region



Tier Identification and Regional Locations

Monitoring Stations

- 1 Daily Use
- 2 Non-daily Use





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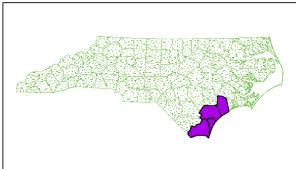
NC Recreation Water Quality Program
Southern Region



Tier Identification and Regional Locations

Monitoring Stations

- 1 Daily Use
- 2 Non-daily Use





Shellfish Sanitation and Recreational Water Quality
June 2021
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A7 - Quality Objectives and Criteria

Primary Objective:

To protect the public health by monitoring the quality of North Carolina's coastal recreational waters and notifying the public when bacteriological standards for safe bodily contact are exceeded.

Quality Objectives:

- To identify swimming areas/beaches and classify them based on human recreational usage.
- To identify monitoring sites that exceed the enterococci geometric mean and singlesample maximum criteria using the Enterolert MPN method for enumeration.
- To evaluate the public health significance of approximately twenty (20) ocean storm drains.
- To document trends in coastal bacteriological water quality.

Measurement Performance Criteria:

Swimming advisory signs are posted and press releases issued for Tier I swimming areas/beaches when a minimum of five (5) samples are collected, equally spaced over 30 days, has a geometric mean of **35 or greater enterococci per 100 mI** or, when both the initial water sample and resample collected exceeds **104 enterococci per 100 mI**. The public is notified via social media release, without an advisory sign when a single sample exceeds **104 enterococci per 100 mI**. for a Tier I site. This is called a pending swimming advisory. A second sample will be collected immediately. If the second sample exceeds **104 enterococci per 100 mI**, the pending swimming advisory is converted into an advisory and the public will be notified by press release, and a sign will be posted.

Rescinding Tier I Advisories

Once the geometric mean exceeds the standard, the swimming advisory is not lifted until two consecutive weekly samples meet the EPA standard of **35 enterococci per 100 ml**. For a geometric mean advisory to be rescinded, the site must have two consecutive good samples,

with good being defined as 35 enterococci per 100 ml or fewer. However, if a situation occurs where two consecutive good samples are recorded, but the geometric mean still exceeds, the advisory will remain in place until the geometric mean falls below 35 enterococci per 100 ml and when two consecutive good samples are recorded concurrently within the same two week period.

Beaches that violate the single-sample maximum criteria for two consecutive tests are re-sampled at the time of the public notification and sign posting. If the re- sample is satisfactory, the advisory may be lifted as soon as 24 hours from the time of the initial advisory notification or posting. If the re-sample is unsatisfactory and the geometric mean is not exceeded, the sign remains posted. If the re-sampling causes the exceedance of the geometric mean, then the geometric mean criteria apply.

The timeframe for posting swimming advisory signs at Tier I beaches, based on the enterococci geometric mean, runs from the beginning of May through the end of September. Weekly sampling of Tier I beaches begins in April of each year so that a running geometric mean is established by May. April and October are considered the shoulder seasons. Seasonal low temperatures during April and October may prevent people from swimming. The project manager determines when advisory signs and public notification are initiated during the shoulder seasons.

Tier II beaches/swimming areas are sampled twice monthly from April to October, with the advisories based entirely on the single sample maximum criteria for two consecutive tests.

For **Tier II sites**, public notification and a swimming advisory sign are posted when both the initial water sample and resample collected exceeds **104 enterococci per 100 ml**. **Tier II** Beaches that violate the criteria are re-sampled at the time of the public notification and sign posting. If this sample exceeds **104 enterococci per 100 ml**, the site is not sampled until the following week. Weekly sampling of the site continues until the enterococci counts are **104**

enterococci per 100 ml or fewer.

A pending swimming advisory, that is public notification via social media release without a sign, takes place when a single sample exceeds **104 enterococci per 100 ml**. If a second sample exceeds **104 enterococci per 100 ml**, the pending swimming advisory, is converted to an advisory and the public is notified, and an advisory sign is posted. Weekly sampling of the site continues until the enterococci counts are **104 enterococci per 100 ml or fewer**.

Other swimming advisories will be posted as precautionary measures when the following activities occur:

- Pumping of floodwaters between the primary dune and the ocean beaches.
- Disposal of dredge material from closed shellfishing waters on ocean beaches.
- Storm drains with discharges into ocean beaches.
- Storm drains that have flow reaching Tier I recreational waters only during wet weather events are posted with permanent advisory signs. The signs read as follows:

WARNING

STORM WATER DISCHARGE AREA

WATERS MAY BE CONTAMINATED BY DISCHARGE FROM PIPE. SWIMMING IS NOT RECOMMENDED WITHIN 200 FT OF THIS SIGN DURING ACTIVE DISCHARGE.

FOR MORE INFORMATION CALL 252 726 6827

OFFICE OF THE STATE HEALTH DIRECTOR

These pipes are also regular monitoring sites. When the bacteriological sampling results for these pipes exceed the standards during active or non-active discharge, bacteriological advisory signs will be placed in addition to the permanent sign. The permanent warning signs

only indicate a risk when storm drains are actively discharging. Adding the bacteriological advisory sign helps to notify the public that testing has indicated bacteria levels that exceed the standards for recreational use, a press release will be issued.

- Storm drains that have continuous active flow that reach ocean recreational waters are posted with permanent advisory signs. The signs read as follows:

WARNING

STORM WATER DISCHARGE AREA

SWIMMING WITHIN 200 FT OF THIS SIGN MAY INCREASE THE RISK OF WATERBORNE ILLNESS.

OFFICE OF THE STATE HEALTH DIRECTOR

These pipes are also regular monitoring sites. When the bacteriological sampling results for these pipes exceed the standards, no bacteriological swimming advisory sign will be posted in addition to permanent sign. This is because the permanent sign indicates a continuous warning.

Swimming advisories are not posted from November through March; however, all monitoring sites are sampled once per month during the non-swimming season.

Public notification and risk communication plan:

The health director or the environmental health supervisor of the local health department is the first to be informed of a swimming advisory. Discussion with the health department determines who the next contact should be, such as a town or county manager. The media are not contacted concerning a swimming advisory until all local and state officials are aware of the situation. The North Carolina Division of Marine Fisheries (NCDMF) then sends out the press release to the Associated Press and local community newspapers at the same time the advisory sign is posted. The press release advisories are aired on TV, radio stations and web sites (see Appendix 5 for press release templates and links to web sites).

Rescinding an advisory follows the same procedures in reverse. The first communication

involves the local health department, then the sign is removed and another press release is issued, declaring the waters are within the swimming standard.

If an advisory sign is needed on the ocean beaches, the sign will be placed on a post or posts at the interface of the wet and dry sand area of the beach. For estuarine waters, the sign may be posted by boat just offshore of the beach or on the shoreline. There may be instances where permission will have to be obtained to post signs on private property, such as on bulkheads or at entrances to marinas. Local Environmental Health Specialists or other local officials may be present when the signs are erected (see Appendix 6 for sign descriptions).

A8 - Special Training Requirements/Certification

SSRWQ laboratory personnel have been instructed in specific health and safety needs as required for employment. The laboratory maintains the required United States FDA and State certifications.

Field personnel are trained in small boat handling and navigation in coastal waters.

Users of GPS equipment must be trained and certified before collecting field data for boundaries and monitoring sites. Personnel are trained in sample collection, transporting samples, recording field data, keying data into the database and following QA/QC protocols.

A9 - Documentation and Records

SSRWQ performs all sample collections and carries responsibility for handling all data collected in the field (see Appendix 7 for an example of the field sampling sheet). The SSRWQ laboratory is responsible for recording the bacteriological data on the field sheet (see Appendix 7). After laboratory staff record the bacteriological data on the field sheet, this information is immediately entered into an Oracle (IBEAM) database. Each region is responsible for entering the analyzed data collected in their area. In the Oracle (IBEAM) database, exceedance limits are set to highlight bacteriological values that exceed the state and federal recreational water quality standards. Regional offices are to report these values to the project manager for issuing public notifications immediately. A back up of this data is recorded in Excel and is maintained at the

main office. The Oracle database has a data exchange node that meets data reporting requirements to the EPA.

Hard copies of the laboratory data, laboratory quality assurance forms, and field sampling data sheets are archived indefinitely at the Morehead City office. Electronic copies of the bacteriological data are backed up and stored on the server in Morehead City, the state's Western Data Center, as well as backup copies stored on compact disc. Sanitary survey reports of recreational waters and any other reports or audits are kept on file in the same manner. Documentation of public notification, original press releases, are filed for one year; however, a spread sheet documenting advisories and rescinds will be filed electronically indefinitely.

SECTION B: MEASUREMENT AND DATA ACQUISITION

B1 - Sampling Process Design

The method for monitoring the 213 sites throughout coastal North Carolina began by grouping the sites in each region to create sampling runs or routes that the staff would travel for a particular day of sample collection. The northern region has three sampling runs consisting of 49 monitoring sites, the central region has eight sampling runs consisting of 101 monitoring sites and the southern region has four sampling runs with 63 monitoring sites. It is common for a sampling run to have a combination of Tier I and II swimming areas. Approximately half of the sampling runs are accessed by boat and half are reached by car and then wading into water from the beach. Weather conditions and tides have a strong influence on choosing a sampling run; therefore, the person collecting the water samples must plan his/her day accordingly. The sampling runs are close enough to one of the three regional laboratories to have water samples in the testing media before 2:00 p.m. each day (see holding time for samples in laboratory QAP Appendix 9).

Tier I beaches that require a minimum of five (5) samples in 30 days are tested using the following schedule:

Northern Region

Sampling Run	Day
Currituck Corolla	Monday
Kitty Hawk to Oregon Inlet	Monday
Ocracoke to Pea Island	Tuesday

Central Region

Sampling Run	Day
Topsail Island	Monday
Bogue Banks	Monday
Ragged Point to Belhaven	Tuesday
Pamlico River	Tuesday
Lower Neuse	Tuesday
Core Sound (Truck)	Wednesday
Bogue Sound	Wednesday
Upper Neuse	Wednesday
Core Sound (Boat)	Thursday

Southern Region

Sampling Run	Day
South Carolina to Holden Beach	Monday
Oak Island to Southport	Tuesday
New Hanover County	Wednesday
Masonboro Sound	Thursday

If the above schedule cannot be met for a particular week, field staff will be responsible for ensuring that five (5) samples in 30 days are collected. This could involve going back to the same monitoring site twice in one week. Tier II swimming areas are sampled primarily by boat twice per month and do not require five (5) samples in 30 days, allowing more flexibility in scheduling the sampling runs.

There are several parameters of interest that are measured at each monitoring site that influence the transport and survival of microorganisms. Data are collected for rainfall, air and water temperature, water depth/sample depth, wind speed and direction, current direction, tidal stage, time of sample collection and salinity. Time of sample collection is critical for determining holding times. The other parameters are for information only and do not affect management

decisions concerning public health. Rainfall data are collected from rain gages scattered throughout the watersheds. Tidal stage and wind speed is determined by personal observation and verified by NOAA weather service. The presence of waterfowl and wildlife in proximity of the monitoring site is also recorded on the field data sheet.

B2 - Sampling Methods

Water samples are collected in 200 mL autoclaved wide mouth glass bottles with the site identification on the lid. Once the water sample is collected, the bottle is tipped to give one (1) inch of air space in the bottle. The water samples are stored immediately on ice in a cooler until all the samples are returned to the laboratory. The six-hour holding time for enterococci samples is not an issue because of the relative proximity of the sampling runs to one of the three laboratories. Quanti-trays with positive wells and plates with colonies are disposed of by placing them in orange biohazard bags and then autoclaving. The autoclaved bags are then taken to the landfill.

Approximately half of the beach monitoring will be accomplished by wading into the surf to collect the sample. When wading, the sampler will use a telescopic golf ball retriever, modified to hold the sample bottle, to reach out approximately 16 feet from the body in kneedeep water to collect the sample. Many of the camps on the coastal rivers and sounds have long piers that extend out over the water. The sample should betaken 6 to 12 inches below the surface of the water at a location along the pier that receives the most use, e.g., ladders, etc. Sampling by boat takes place in approximately three feet of water with the sample collected 12 inches below the surface. A stainless steel rod with a sample holder will be used to collect the sample from the boat. Sampling personnel should avoid disturbing bottom sediment in either approach, to collecting the sample.

Dare County has eight ocean storm drains that extend to the water's edge at low tide.

The mouths of these storm drains are partially or completely submerged at high tide. Samples are to be collected approximately 10 feet to either side of the Dare County drains when

practical. At times, surf conditions may not be safe to be within 10 feet of the pipe. The water sample collected at the Hanby Beach storm drain in New Hanover County will be sampled in the same manner. The remaining storm drains in New Hanover, Brunswick and Carteret counties do not extend to the water's edge. These drains are sampled where the swash enters the surf. The water depth for sample collection at all storm drains is the same as the other monitor sites in the surf, just below the surface in approximately knee- deep water.

It may be necessary at some sites that exceed the geometric mean protocol to conduct additional sampling to define the extent of the pollution. Once the advisory sign is posted, sampling may be initiated at a point 200 feet on either side of the sign. The program manager determines when, if any, additional sampling will be conducted.

Personal watercraft rental sites in most cases are classified as Tier II sites. The samples are collected in the area of the sound where the renters are allowed to ride. This is usually a sectioned-off area marked by buoys. If an advisory is needed, the sign will be posted near shore where watercraft users can see the sign before entering the riding area.

B3 – Sample Handling and Custody

The sample collectors are responsible for ensuring the samples are stored and handled properly while in the field. The samples are stored immediately on ice in a cooler to chill the sample and to limit the exposure to UV light. The time is recorded on the field-sampling sheet for each sample collected. The six-hour holding limit for enterococci is not a factor because each sampling run can be completed with samples back to one of the three laboratories within 3 to 4 hours. Laboratory personnel are responsible for recording the time on data sheets when samples are planted into the media and the times samples can be analyzed after incubation (see Appendix 9 for laboratory QA). Both laboratory and field personnel are responsible for signing off on the chain of custody checklist on the backside of the field-sampling sheet (see Appendix 7, page 2).

B4 - Analytical Methods

Using the Idexx Enterolert method and EPA method 1600 are discussed in the laboratory quality assurance plan. A failure in any part of the laboratory procedure results in the collection of another sample. Laboratory staff members are responsible for ensuring that the project manager receives the bacteriological results immediately upon completion of the analysis, and correcting any laboratory procedures that may occur. The laboratory staff members are: KC McAvoy in Nags Head, Amanda Hewes in Morehead City, and Holly White in Wilmington.

B5 - Quality Control

A sample is collected in the field and labeled temperature control that is measured upon arrival to the laboratory. The laboratory refuses samples that are above the temperature at which they were collected and samples that exceed holding time. Split samples are also taken at different frequencies and shared among the three labs to compare results. During laboratory analysis, a pure culture of *Enterococcus faecium* is used as a positive control and *Serratia marcescens* is used as the negative control.

The acceptance criteria for enterococci are based upon the MPN table provided by Idexx Laboratories. The smallest number of enterococci that can be analyzed is 9 organisms per 100 ml. The highest density of enterococci that can be analyzed is 2005 organisms per 100 ml. Higher densities can be analyzed by further diluting the sample. The critical value of enterococci is 104 organisms per 100 ml for single samples exceedances, are well within the capabilities of the Enterolert method. Idexx reports that Enterolert has a false negative rate 0.4% and a false positive rate of 5.1%.

Other analytical controls are detailed in the laboratory QAP in Appendix 9. Technicians at each regional office review each other's data entry for mistakes and random data entry reviews are conducted in the main office for quality control purposes.

B6 – Instrument /Equipment Testing, Inspection, and Maintenance

The two major equipment items needed to do the recreational water quality method are an autoclave and an air incubator. A maintenance contract with the autoclave manufacturer requires that the autoclave have preventive maintenance once every two months by authorized technicians.

Laboratory personnel check the autoclave monthly for sterility. Air incubators are checked twice daily for proper temperatures using a certified thermometer.

B7 - Instrumentation Calibration and Frequency

Laboratory equipment is routinely inspected and calibrated at different times of the year to meet FDA and State certification requirements. The only field instruments that need calibrating are refractometers and thermometers. These two instruments are calibrated against known standards.

B8 - Inspection/acceptance of Supplies and Consumables

Lab supplies are ordered through major scientific supply companies and inspected upon receipt by the project manager. All of the growth mediums and reagents are sterile upon arrival and received in sealed packaging. Each package has a certificate of sterility by the manufacturer.

The supplies will be returned to manufacturer if a seal is broken on any of the packaging. Field personnel inspect field supplies before leaving the office each day.

B9 – Non-direct Measurements

Tide tables are the only non-direct measurements that will be used in the project. Tide tables are often used when planning sampling runs by boat. Storms and wind can cause delays or early arrival in the actual tidal stage but are not a critical issue to the project.

B10 – Data Management

The data is currently being entered into an Integrated Build Environment & Application

Management (IBEAM) database, which is accessed for public use through our program

website: http://portal.ncdenr.org/web/mf/rwg-sampling-data

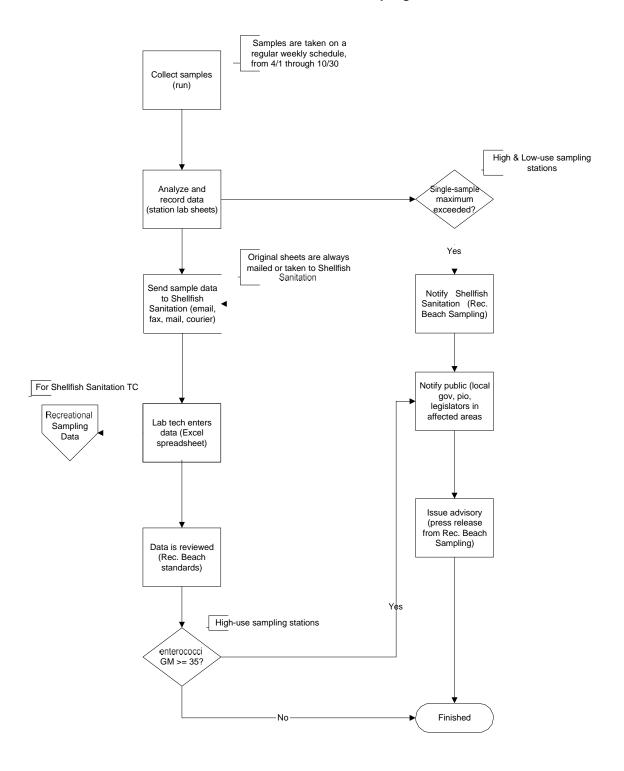
The IBEAM database is web-enabled and has both numerical and **Pr**ogram tracking, beach **A**dvisories, **W**ater quality standards, and **N**utrients (PRAWN) data.

Data is entered into the IBEAM database at each regional office and is stored in an ORACLE database housed in Raleigh, NC which is backed up nightly. All of the data from each region is entered into the Excel spread sheet from staff at the Morehead City Office for redundancy. Entries from the spreadsheet and the IBEAM database are compared to one another as a check (see Appendix 7). When data entered into IBEAM from the Northern Region and Southern Region does not match with the Central Region spread sheet, the data entry staff are required to find and correct the mistake. Advisories are not posted until both databases can verify the geometric mean. The project manager reviews the database daily to look at the geometric mean data and orders the posting of advisory signs and the issue of press releases accordingly.

All of the data on the server have timed backups that are stored on tape. Redundancy in data entry prevents incorrect data from being stored on the tapes. Current copies of the data are also available on employees' desktop computers.

The following flow chart shows the process by which the data is generated and how the data is used.

Data Generation Routine Beach Sampling



SECTION C: ASSESSMENT AND OVERSIGHT

C1 - Assessments and Response Actions

As Section Chief, Shannon Jenkins has oversight for the shellfish and beach monitoring programs. Erin Bryan-Millush is the Beach Monitoring Project Manager and is responsible for regularly reviewing the progress of the project, compiling data and supervising employees.

The project manager is responsible for posting swimming advisory signs, directing public notification activities, and visiting the field offices regularly to assure adherence to the quality assurance project plan. Performance reviews are scheduled every six months for individual employee assessments. The project manager is responsible for any corrective action needed to ensure that the staff in the recreational water quality program is adhering to the QAPP and program objectives. The program manager is responsible for notification of health departments, local, state and federal governments as well as interest groups and the public; analyzing and preparing data for submittal to the Environmental Protection Agency and public outreach and education about the program.

The SSRWQ staff monitors the documentation of laboratory/field procedures and data analysis for their specific region throughout the beach-monitoring project.

C2 – Reports to Management

Data entry personnel are responsible for submitting data summaries to the project manager daily. Field and lab staff members are responsible for reporting quality assurance issues as they occur to the project manager. Individual reports for daily objectives and accomplishments are available for management at any time. The project manager reports to the EPA all swimming advisories as they are issued. An in-depth survey of the beach monitoring is reported to the EPA in January of each year.

SECTION D: DATA VALIDATION AND USABILITY

D1 - Data Review, Verification, and Validation

Each region has standardized field and laboratory procedures. Sample collection, handling of samples and lab analysis are all conducted in the same manner. Splitting samples and sharing them with each of the three labs to analyze helps verify that protocols are followed properly. Bacteriological data that is derived from samples where quality assurance is questionable will be rejected. The regional laboratories are state- and FDA-certified.

D2 - Verification and Validation Methods

Verification and validation are conducted by the staff members who record and enter the data. At least two people are involved in the laboratory analysis to ensure that samples are read and recorded correctly on to the field/lab form. The laboratory staff responsible for resolving laboratory issues are: KC McAvoy and a field technician in Nags Head, Amanda Hewes, Wayne Hall and a field technician in Morehead City, Holly White and a field technician in Wilmington. The Project Manager and Quality Assurance Officer, Erin Bryan-Millush, will assist in possible issue resolution.

Controls are setup to verify that samples are being read properly (see Sect. B-5 Quality Control). The laboratory results are entered into the database by technicians. Two technicians at each regional office are responsible for ensuring the data is entered into the system free of mistakes. Each technician s work is reviewed by the other technician for 100% verification. The project manager performs random spot checks on 5% of each office s data handling. The database is set up so that data summaries are verified by having the program to automatically calculate geometric means.

D3 – Reconciliation with User Requirements

The geometric means and single sample maximums are compared to the standards in the EPA's Beach Guidance manual for posting swimming advisories.

<u>APPENDIX</u> 1: List of Tier One Monitoring Sites Funded by EPA Grant with sampling frequency and the single sample maximum.

SITE	COUNTY	Tier	LOCATION	SS Max	EPA_ID	SEASON	SEASON_ FREQUENCY	SHOULDER_SEASON FREQUENCY	OFF_SEASON FREQUENCY
C115					_	4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
A	BEAUFORT	1	sound	104	NC442154	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
N87	BEAUFORT	1	sound	104	NC483929	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S1	BRUNSWICK	1	ocean	104	NC499745	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S2	BRUNSWICK	1	ocean	104	NC758778	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S3	BRUNSWICK	1	ocean	104	NC848108	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S5A	BRUNSWICK	1	ocean	104	NC379310	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S5B	BRUNSWICK	1	ocean	104	NC442815	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S5	BRUNSWICK	1	ocean	104	NC175823	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S6A	BRUNSWICK	1	ocean	104	NC971811	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S6B	Brunswick	1	ocean	104	NC427139	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S6C	BRUNSWICK	1	ocean	104	NC164372	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S6	BRUNSWICK	1	ocean	104	NC463026	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S9A	BRUNSWICK	1	ocean	104	NC975034	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S9B	BRUNSWICK	1	ocean	104	NC345154	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S9C	BRUNSWICK	1	ocean	104	NC140790	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S9D	BRUNSWICK	1	ocean	104	NC861543	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S9	BRUNSWICK	1	ocean	104	NC576773	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S10A	BRUNSWICK	1	ocean	104	NC998441	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S10B	BRUNSWICK	1	ocean	104	NC642326	9/30	Days	month	month
					-	4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S10C	BRUNSWICK	1	ocean	104	NC521331	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S10D	BRUNSWICK	1	ocean	104	NC892936	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S10E	BRUNSWICK	1	ocean	104	NC654550	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S10F	BRUNSWICK	1	ocean	104	NC457651	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S10G	BRUNSWICK	1	ocean	104	NC517459	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S10	BRUNSWICK	1	ocean	104	NC698990	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S11A	BRUNSWICK	1	ocean	104	NC187482	9/30	Days	month	month
		-		1		4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S13A	BRUNSWICK	1	ocean	104	NC894449	9/30	Days	month	month
		-		1		4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
	BRUNSWICK		ocean	104	NC384247	9/30	Days	month	month

ĺ			I	I	l	4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S13C	BRUNSWICK	1	ocean	104	NC404663	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S13	BRUNSWICK	1	ocean	104	NC873506	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S14A	BRUNSWICK	1	ocean	104	NC742373	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S14B	BRUNSWICK	1	ocean	104	NC143620	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S15A	BRUNSWICK	1	ocean	104	NC497594	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S15	BRUNSWICK	1	ocean	104	NC449749	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S16A	BRUNSWICK	1	sound	104	NC569455	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S16B	BRUNSWICK	1	ocean	104	NC191050	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S16C	BRUNSWICK	1	ocean	104	NC895384	9/30	Days	month	month
	~					4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
C2	CARTERET	1	ocean	104	NC643293	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
C3A	CARTERET	1	ocean	104	NC345060	9/30	Days	month	month
-	G A D THE D FITT			101		4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
C3	CARTERET	l	ocean	104	NC895537	9/30	Days	month	month
C14	CARTERET	1		104	NG2 42007	4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
C4A	CARTERET	1	ocean	104	NC343007	9/30	Days	month	month
CAD	C A DEED FEE			104	NGGGGGG	4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
C4B	CARTERET	1	ocean	104	NC737019	9/30	Days	month	month
C4	CADTEDET	1		104	NC714612	4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
C4	CARTERET	1	ocean	104	NC714613	9/30 4/1-	Days	month	month
C5 A	CARTERET	1		104	NC152475	9/30	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
C5A	CARTERET	1	ocean	104	NC152475	4/1-	Days 5 in 30	month 10/1-10/31 - Twice per	month 11/1-3/3 - Once per
C5	CARTERET	1	00000	104	NC115357	9/30	Days	month	month
CJ	CARTERET	1	ocean	104	110113337	4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
C6A	CARTERET	1	ocean	104	NC102958	9/30	Days	month	month
COA	CARTERET	1	ocean	104	110102936	4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
C6	CARTERET	1	ocean	104	NC120547	9/30	Days	month	month
	CHRIEREI	1	occan	104	110120547	4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
C7	CARTERET	1	ocean	104	NC244236	9/30	Days	month	month
	CHRIEREI	-	occur	101	110211230	4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
C8	CARTERET	1	ocean	104	NC147416	9/30	Days	month	month
	CHATEREST	-	o count	10.	1,011,7110	4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
C9	CARTERET	1	ocean	104	NC475791	9/30	Days	month	month
		-		1		4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
C10A	CARTERET	1	ocean	104	NC952661	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
C10B	CARTERET	1	ocean	104	NC511988	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
C55A	CARTERET	1	sound	104	NC189579	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
C57	CARTERET	1	sound	104	NC101248	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
C69A	CARTERET	1	sound	104	NC888920	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
C69C	CARTERET	1	ocean	104	NC106127	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
N1A	CURRITUCK	1	ocean	104	NC109355	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
3.74	CURRITUCK	1	ocean	104	NC790915	9/30	Days	month	month
N1	COMMITTER								
N1 N2	CURRITUCK				NC856780	4/1- 9/30	5 in 30 Days	10/1-10/31 - Twice per	11/1-3/3 - Once per

N4 (1 N5A 1 N7A 1 N7 1 N12A 1	CURRITUCK CURRITUCK DARE DARE	1	ocean	104	NC542433	9/30 4/1-	Days 5 in 30	month 10/1-10/31 - Twice per	month 11/1-3/3 - Once per
N5A 1 N7A 1 N7 1 N12A 1	DARE		ocean	104					1 1/1 = 1/1 = V / HUSE DEL
N5A 1 N7A 1 N7 1 N12A 1	DARE	1			NC846710	9/30	Days	month	month
N7A I N7 I N12A I		1				4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
N7A I N7 I N12A I	DARE		ocean	104	NC995692	9/30	Days	month	month
N7 I N12A I	DARE					4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
N12A		1	ocean	104	NC658738	9/30	Days	month	month
N12A						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
	DARE	1	ocean	104	NC653898	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
N12C	DARE	1	ocean	104	NC992884	9/30	Days	month	month
N12C					NC332962	4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
	DARE	1	ocean	104		9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
N12 I	DARE	1	ocean	104	NC933106	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
N14A I	DARE	1	ocean	104	NC148512	9/30	Days	month	month
	D + D =			101	37G406FF0	4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
N14 I	DARE	1	ocean	104	NC196750	9/30	Days	month 10/1-10/31 - Twice per	month
N115 1	DARE	1		104	NC701052	4/1- 9/30	5 in 30		11/1-3/3 - Once per
N15 I	DARE	1	ocean	104	NC701853	4/1-	Days 5 in 30	month 10/1-10/31 - Twice per	month 11/1-3/3 - Once per
N16A I	DARE	1	ocean	104	NC297658	9/30	Days	month	month
NIOA	DAKE	1	ocean	104	140297036	4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
N16B I	DARE	1	ocean	104	NC669365	9/30	Days	month	month
1110D	DINC	- 1	occur	104	11007303	4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
N16C I	DARE	1	ocean	104	NC845140	9/30	Days	month	month
11100 1	DINE	•	occum	101	110013110	4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
N16D I	DARE	1	ocean	104	NC706960	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
N16 1	DARE	1	ocean	104	NC952496	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
N17A I	DARE	1	ocean	104	NC703962	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
N17B I	DARE	1	ocean	104	NC194415	9/30	Days	month	month
	D + D =			101	270171077	4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
N17C I	DARE	1	ocean	104	NC174377	9/30	Days	month	month
N117 I	DARE	1		104	NG(75200	4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
N17 I	DARE	1	ocean	104	NC675298	9/30 4/1-	Days 5 in 30	month 10/1-10/31 - Twice per	month 11/1-3/3 - Once per
N18 I	DARE	1	00000	104	NC888506	9/30	Days	month	month
1010	DAKE	1	ocean	104	110000300	4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
N19A I	DARE	1	ocean	104	NC325364	9/30	Days	month	month
1,1/11 1	2711112		550411	10-	110323304	4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
N22 I	DARE	1	ocean	104	NC239137	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
N23 I	DARE	1	ocean	104	NC570729	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
N25 I	DARE	1	ocean	104	NC944159	9/30	Days	month	month
		_				4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
N26B 1	DARE	1	ocean	104	NC144418	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
N26 I	DARE	1	ocean	104	NC289380	9/30	Days	month	month
NOT .	DARE			101	NOSCOLO	4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
N27 I	DARE	1	ocean	104	NC566464	9/30	Days	month	month
NAOF .	DARE			101	NG001017	4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
N29B I	DARE	1	ocean	104	NC991015	9/30	Days	month	month
N20	DADE	1	00000	104	NC005002	4/1- 9/30	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
N29 I	DARE	1	ocean	104	NC995992	4/1-	Days 5 in 30	month 10/1-10/31 - Twice per	month 11/1-3/3 - Once per
			ocean	104	NC440580	9/30	Days	month	month

		1				4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
N32	DARE	1	ocean	104	NC927135	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
N34	DARE	1	ocean	104	NC422633	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
N37	DARE	1	ocean	104	NC189209	9/30	Days	month	month
N/20	DARE	1		104	NG200267	4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
N39	DARE	1	ocean	104	NC380367	9/30 4/1-	Days 5 in 30	month 10/1-10/31 - Twice per	month
N40	DARE	1	ocean	104	NC560974	9/30	Days	month	11/1-3/3 - Once per month
1140	DAKE	1	ocean	104	NC300974	4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
N85A	DARE	1	ocean	104	NC635010	9/30	Days	month	month
	Dine	1	- Ocean	10.	1,0000010	4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
N85	DARE	1	ocean	104	NC524248	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
N88	DARE	1	sound	104	NC981058	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
N91	DARE	1	sound	104	NC952532	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
N41A	HYDE	1	ocean	104	NC709868	9/30	Days	month	month
T 4 1	HWDE	1		104	NG260124	4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
N41	HYDE	1	ocean	104	NC368134	9/30	Days	month	month
N42	HYDE	1	00000	104	NC562881	4/1- 9/30	5 in 30 Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
N42	HIDE	1	ocean	104	NC302881	4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
N43	HYDE	1	ocean	104	NC318235	9/30	Days	month	month
173	NEW	1	occan	104	140310233	4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S18B	HANOVER	1	ocean	104	NC918299	9/30	Days	month	month
лов	NEW	-	occur	101	1(0)102)	4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S18C	HANOVER	1	ocean	104	NC274681	9/30	Days	month	month
	NEW					4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S18D	HANOVER	1	ocean	104	NC301060	9/30	Days	month	month
	NEW					4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S18	HANOVER	1	ocean	104	NC593669	9/30	Days	month	month
	NEW					4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S19B	HANOVER	1	ocean	104	NC765666	9/30	Days	month	month
	NEW					4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S19C	HANOVER	1	ocean	104	NC796965	9/30	Days	month	month
710	NEW	1		104	NG520102	4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S19	HANOVER	1	ocean	104	NC530102	9/30	Days 5 in 30	month 10/1-10/31 - Twice per	month
S20A	NEW HANOVER	1	00000	104	NC616697	9/30	Days	month	11/1-3/3 - Once per month
520A	NEW	1	ocean	104	NC010097	4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S21B	HANOVER	1	ocean	104	NC939344	9/30	Days	month	month
,	NEW	1	550411	107	110,0,0,0	4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S22A	HANOVER	1	ocean	104	NC375708	9/30	Days	month	month
	NEW	1				4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S22B	HANOVER	1	sound	104	NC748601	9/30	Days	month	month
	NEW					4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S22C	HANOVER	1	sound	104	NC252230	9/30	Days	month	month
	NEW	1				4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S22E	HANOVER	1	sound	104	NC230511	9/30	Days	month	month
	NEW					4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S22F	HANOVER	1	ocean	104	NC349062	9/30	Days	month	month
1000	NEW		,	104	NG771501	4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S22G	HANOVER	1	sound	104	NC771581	9/30	Days	month	month
2024	NEW	1	0.000	104	NOTTOTEO	4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S23A	HANOVER	1	ocean	104	NC773758	9/30	Days	month	month
S23B	NEW	1	ocean	104	NC632805	4/1- 9/30	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
. / 113	HANOVER	1	ocean	104	110032003		Days	month 10/1-10/31 - Twice per	month
J23B	NEW					4/1-	5 in 30		11/1-3/3 - Once per

	NEW		ĺ			4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S48A	HANOVER	1	sound	104	NC547001	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
C13	ONSLOW	1	ocean	104	NC577316	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S29A	ONSLOW	1	ocean	104	NC266219	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S29C	ONSLOW	1	ocean	104	NC440062	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S30A	ONSLOW	1	ocean	104	NC741107	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S30C	ONSLOW	1	ocean	104	NC645929	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S30E	ONSLOW	1	ocean	104	NC899473	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S30F	ONSLOW	1	ocean	104	NC471307	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S30	ONSLOW	1	ocean	104	NC865785	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S25A	PENDER	1	sound	104	NC467377	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S28B	PENDER	1	ocean	104	NC403790	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S28C	PENDER	1	ocean	104	NC947796	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S28	PENDER	1	ocean	104	NC098150	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S29B	PENDER	1	ocean	104	NC594897	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
S29	PENDER	1	ocean	104	NC526485	9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
C51C	CARTERET	1	sound	104		9/30	Days	month	month
						4/1-	5 in 30	10/1-10/31 - Twice per	11/1-3/3 - Once per
C48C	CARTERET	1	sound	104	NC727802	9/30	Days	month	month
2.100	C. IICTERET		Sound	107	1.0/2/002	7/30	Dujo	o.itti	monui

<u>APPENDIX 2</u>: List of Tier II Monitoring Sites Funded by State of North Carolina with sampling frequency and the single sample maximum.

SITE	COUNTY	TIER	LOCATION	SSMax	EPA_ID	SEASON	SEASON_FREQUENCY	SHOULDER_SEASON_FREQUENCY	OFF_SEASON_FREQUENCY
С7В	CARTERET	2	sound	104	NC429821	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C22A	ONSLOW	2	sound	104	NC930678	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C23B	ONSLOW	2	sound	104	NC611568	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C25	ONSLOW	2	sound	104	NC499511	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C27A	ONSLOW	2	sound	104	NC892318	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C30A	CARTERET	2	sound	104	NC377628	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C30	CARTERET	2	sound	104	NC254979	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C31A	CARTERET	2	sound	104	NC615139	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C33A	CARTERET	2	sound	104	NC927355	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C34A	CARTERET	2	sound	104	NC456163	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C35A	CARTERET	2	sound	104	NC722475	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C36	CARTERET	2	sound	104	NC623291	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month

C39A	CARTERET	2	sound	104	NC272309	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C40A	CARTERET	2	sound	104		4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C40	CARTERET	2	sound	104	NC320933	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C47A	CARTERET	2	sound	104	NC411851	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C47B	CARTERET	2	sound	104	NC160448	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C48B	CARTERET	2	sound	104	NC135340	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C51B	CARTERET	2	sound	104	NC958671	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C51	CARTERET	2	sound	104	NC659044	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C53A	CARTERET	2	sound	104	NC852484	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C54A	CARTERET	2	sound	104	NC907794	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C55B	CARTERET	2	sound	104	NC381539	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C56A	CARTERET	2	sound	104	NC730320	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C56	CARTERET	2	sound	104	NC179171	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C58	CARTERET	2	sound	104	NC997135	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C59A	CARTERET	2	sound	104	NC380630	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C60A	CARTERET	2	sound	104	NC935797	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C62	CARTERET	2	sound	104	NC951761	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C64	CARTERET	2	sound	104	NC341418	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C65A	CARTERET	2	sound	104	NC852895	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C66	CARTERET	2	sound	104	NC626501	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C69D	CARTERET	2	sound	104	NC347773	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C75A	CARTERET	2	sound	104	NC861560	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C81A	CARTERET	2	sound	104	NC434812	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C81B	CARTERET	2	sound	104	NC134999	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C85	CARTERET	2	sound	104	NC586930	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C89A	PAMLICO	2	sound	104	NC624353	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C90A	PAMLICO	2	sound	104	NC990224	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C92A	PAMLICO	2	sound	104	NC155524	4/1-9/30	5 in 30 Days	2x Monthly	11/1-3/3 - Once per month
C92	PAMLICO	2	sound	104	NC174861	4/1-9/30	5 in 30 Days	2x Monthly	11/1-3/3 - Once per month
C93	PAMLICO	2	sound	104	NC854048	4/1-9/30	5 in 30 Days	2x Monthly	11/1-3/3 - Once per month
C94	PAMLICO	2	sound	104	NC490467	4/1-9/30	5 in 30 Days	2x Monthly	11/1-3/3 - Once per month
C95	PAMLICO	2	sound	104	NC902753	4/1-9/30	5 in 30 Days	2x Monthly	11/1-3/3 - Once per month
C96A	PAMLICO	2	sound	104	NC589816	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C99	CRAVEN	2	sound	104	NC461235	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C100A	CRAVEN	2	sound	104	NC558811	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C104	CRAVEN	2	sound	104	NC193701	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C105B	CRAVEN	2	sound	104	NC821771	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C106A	CRAVEN	2	sound	104	NC905913	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month

C107	CRAVEN	2	sound	104	NC619539	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C110A	CRAVEN	2	sound	104	NC608977	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C111A	BEAUFORT	2	sound	104	NC105938	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C111	BEAUFORT	2	sound	104	NC556462	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C112A	BEAUFORT	2	sound	104	NC482470	4/1-9/30	5 in 30 Days	2x Monthly	11/1-3/3 - Once per month
C114	PAMLICO	2	sound	104	NC659798	4/1-9/30	5 in 30 Days	2x Monthly	11/1-3/3 - Once per month
C117A	BEAUFORT	2	sound	104	NC968346	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C118	BEAUFORT	2	sound	104	NC778708	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C119A	BEAUFORT	2	sound	104	NC575571	4/1-9/30	5 in 30 Days	2x Monthly	11/1-3/3 - Once per month
C120A	BEAUFORT	2	sound	104	NC808817	4/1-9/30	5 in 30 Days	2x Monthly	11/1-3/3 - Once per month
C126A	BEAUFORT	2	sound	104	NC635491	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
N29A	DARE	2	sound	104	NC347842	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
N39A	DARE	2	sound	104	NC807801	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
S30D	ONSLOW	2	sound	104	NC187979	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
S35A	BRUNSWICK	2	sound	104	NC451008	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
S37	BRUNSWICK	2	sound	104	NC803771	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
S43A	BRUNSWICK	2	sound	104	NC642348	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
S45A	NEW HANOVER	2	sound	104	NC853733	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
S45	NEW HANOVER	2	sound	104	NC813450	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
S48	NEW HANOVER	2	sound	104	NC519384	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
S54A	ONSLOW	2	sound	104	NC431213	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
S54	PENDER	2	sound	104	NC215656	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
S68	NEW HANOVER	2	sound	104		4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
S69	NEW HANOVER	2	sound	104		4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
S70	NEW HANOVER	2	sound	104		4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month

APPENDIX 4: Station Locations

STATION	LATITUDE	LONGITUDE	STATION	LATITUDE	LONGITUDE	STATION	LATITUDE	LONGITUDE
Central Region			Northern Region			Southern Region		
C2	34.69394638	-76.69724201	N1A	36.43585876	-75.83968249	S1	33.86356188	-78.51527162
C3A	34.69700737	-76.72403037	N1	36.39663574	-75.82791646	S2	33.86657985	-78.50650485
C3	34.69583086	-76.71178281	N2	36.37644043	-75.82329652	S3	33.87140195	-78.48822958
C4A	34.69664188	-76.7809633	N3	36.32844831	-75.8094266	S5A	33.87876782	-78.46523232
C4B	34.69494278	-76.80578492	N4	36.2787749	-75.79195055	S5B	33.88302916	-78.44991014
C4	34.69731061	-76.73911912	N5A	36.19613529	-75.75606546	S5	33.88626276	-78.43631643
C5A	34.69084475	-76.84535789	N7A	36.12166838	-75.72180795	S6A	33.89445128	-78.40493636
C5	34.69293202	-76.82888335	N7	36.14536226	-75.73410635	S6B	33.8897331	-78.42605463

C6A	34.68284924	-76.9069427	N12A	36.04417804	-75.67403845	S6C	33.89770606	-78.39411407
C6	34.68690932	-76.88116866	N12C	36.021895	-75.659347	S6	33.89277787	-78.41128612
С7В	34.68640917	-76.9098865	N12	36.06682161	-75.68917936	S9A	33.90618264	-78.34359023
C7	34.67524399	-76.95655585	N14A	36.01420467	-75.65463709	S9B	33.90753083	-78.33264435
C8	34.66073634	-77.03419145	N14	36.01566147	-75.65553878	S9C	33.91440851	-78.24638171
C9	34.65548359	-77.05547456	N15	35.99848263	-75.64574377	S9D	33.91007377	-78.30708153
C10A	34.65419598	-77.06031474	N16A	35.97131922	-75.63000341	S9	33.91058882	-78.29699199
C10B	34.64475863	-77.08942269	N16B	35.987269	-75.639445	S10A	33.91370788	-78.19015525
C13	34.63316855	-77.13784688	N16C	35.981365	-75.635893	S10B	33.91385405	-78.15849676
C22A	34.65263906	-77.16531317	N16D	35.967732	-75.628239	S10C	33.91422538	-78.17983866
C23B	34.62592827	-77.17088553	N16	35.98904924	-75.64023789	S10D	33.91428501	-78.17192491
C25	34.63982813	-77.13963815	N17A	35.95880885	-75.62327886	S10E	33.91389885	-78.20595714
C27A	34.68483268	-77.12323518	N17B	35.962546	-75.625329	S10F	33.91360537	-78.2137974
C30A	34.653	-77.102	N17C	35.960276	-75.624243	S10G	33.91284741	-78.22805586
C30	34.67275548	-77.10026206	N17	35.96544073	-75.62690244	S10	33.91393669	-78.2605221
C31A	34.67982058	-77.06532832	N18	35.9315153	-75.60799394	S11A	33.9134154	-78.26723028
C33A	34.66383475	-77.06795354	N19A	35.91036276	-75.59617157	S13A	33.90945097	-78.11682515
C34A	34.67360202	-77.00797809	N22	35.846914	-75.56304911	S13B	33.9080251	-78.10693851
C35A	34.686	-77.052	N23	35.7986653	-75.54036219	S13C	33.91156206	-78.13334012
C36	34.69660735	-77.00918628	N25	35.74029911	-75.50219232	S13	33.91272275	-78.14684113
C39A	34.70469152	-76.96295099	N26B	35.6086426	-75.46491865	S14A	33.90324283	-78.08117223
C40A	34.702	-76.823	N26	35.68026788	-75.47987822	S14B	33.90563435	-78.09105227
C40	34.72365189	-76.89543785	N27	35.58499122	-75.46100908	S15A	33.89165045	-78.03533021
C47A	34.70909932	-76.74541518	N29A	35.53366568	-75.47583433	S15	33.89616568	-78.05453433
C47B	34.71695021	-76.74687376	N29B	35.43844176	-75.48355779	S16A	33.88092603	-77.9978067
C48B	34.7223024	-76.75529379	N29	35.53145704	-75.4680156	S16B	33.85655697	-77.99993404
C48C	34.719	-76.724	N30	35.37705603	-75.49214639	S16C	33.84994935	-77.95970035
C51B	34.71779444	-76.71602039	N32	35.32189827	-75.50682384	S18B	33.99032437	-77.90864444
C51C	34.722	-76.747	N34	35.25404181	-75.52084915	S18C	33.99347899	-77.90761908
C51	34.72189453	-76.75116269	N37	35.23213845	-75.60439615	S18D	33.979636	-77.912031
C53A	34.72161621	-76.68715455	N39A	35.221141	-75.657925	S18	33.96407925	-77.92111895
C54A	34.70926372	-76.67636051	N39	35.22352804	-75.64332968	S19B	34.0119747	-77.89960854
C55A	34.728	-76.668	N40	35.20439656	-75.70266471	S19C	34.03131962	-77.89204232
C55B	34.72273139	-76.66801969	N41A	35.1578687	-75.84206461	S19	33.99806545	-77.90506805
C56A	34.70972225	-76.63204321	N41	35.18077289	-75.77833293	S20A	34.05247005	-77.88310913
C56	34.71488538	-76.6626949	N42	35.12323539	-75.92108097	S21B	34.06373015	-77.87869288
C57	34.71368384	-76.68021494	N43	35.1023641	-75.95907669	S22A	34.19308497	-77.80444986
C58	34.68723685	-76.64406304	N85A	36.00594398	-75.64988082	S22B	34.19639699	-77.80541608

C59A	34.67938603	-76.61910806	N85	36.01151804	-75.6530312	S22C	34.20391228	-77.79942986
C60A	34.69922408	-76.65151301	N87	35.53305101	-76.61257371	S22E	34.20169548	-77.80127128
C62	34.78922414	-76.60785984	N88	35.95228739	-75.63296435	S22F	34.20732533	-77.79409262
C64	34.71390843	-76.5788927	N91	36.01837068	-75.72686409	S22G	34.1942071	-77.8074981
C65A	34.70213019	-76.52039585				S23A	34.23375944	-77.77398669
C66	34.684697	-76.528839				S23B	34.22623357	-77.78119418
C69A	34.62393278	-76.52515449				S23	34.21386241	-77.78829205
C69C	34.62126555	-76.52076971				S25A	34.35025327	-77.65349171
C69D	34.620872	-76.551299				S28B	34.3893186	-77.59791736
C75A	34.76136856	-76.41306228				S28C	34.35018617	-77.64663866
C81A	35.0183845	-76.31227876				S28	34.36481114	-77.62854856
C81B	35.01925089	-76.31549635				S29A	34.46037931	-77.48462902
C85	34.98	-76.596				S29B	34.44117942	-77.51882273
C89A	35.02698691	-76.68830774				S29C	34.47525938	-77.45583558
C90A	35.00897073	-76.70949719				S29	34.42419862	-77.54583075
C92A	34.99274117	-76.75734228				S30A	34.49577289	-77.41161113
C92	34.99526013	-76.7524396				S30C	34.50980464	-77.38126226
C93	34.97097118	-76.79361553				S30D	34.49718715	-77.42823821
C94	34.96662672	-76.79839251				S30E	34.51987886	-77.35868008
C95	34.99261474	-76.85546497				S30F	34.49934837	-77.40462231
C96A	35.02378442	-76.90118418				S30	34.49169917	-77.42103306
C99	35.0646135	-76.97017789				S35A	33.91338647	-78.33582797
C100A	35.10307527	-77.03494406				S37	33.90336872	-78.39430835
C104	34.98458069	-76.94728472				S43A	33.91731846	-78.01610671
C105B	34.95276437	-76.89564438				S45A	34.05787965	-77.88872179
C106A	34.91765438	-76.85336357				S45	34.05201506	-77.91788558
C107	34.94159687	-76.82645276				S48A	34.18760111	-77.81363808
C110A	34.930135	-76.791425				S48	34.18308545	-77.81912255
C111A	35.53559212	-77.04134147				S54A	34.46965981	-77.50848406
C111	35.53510588	-77.05028196				S54	34.43076997	-77.55032884
C112A	35.51387771	-77.00994522				S68	34.081	-77.882
C114	35.18037399	-76.66325786				S69	34.159	-77.849
C115A	35.45970011	-76.90123053				S70	34.22	-77.809
C117A	35.43516185	-76.90837622						
C118	35.44854314	-76.92409325						
C119A	35.44135996	-76.9490002						
C120A	35.4754323	-76.99484537						
C126A	35.47781425	-76.93127503						

APPENDIX 5: Press release templates

Roy Cooper

Governor

John Nicholson

Interim Secretary, DEQ

Release: Immediate C
Date: P



Kathy B. Rawls, Division Director

Contact: <u>Erin Bryan-Millush</u> Phone: 252-808-8153

Water quality swimming advisory issued for (sound-side/ocean-side) site in X County

MOREHEAD CITY – An advisory against swimming was posted today at a(n) (sound-side/ocean-side) site in X County, where state officials found bacteria levels in the water that exceed the state's and Environmental Protection Agency's recreational water quality standards.

The advisory is for **PUBLIC DESCRIPTION** in **TOWN.** Test results of water samples taken on **DATE** and **DATE** indicate bacteria levels that exceed the state and federal action levels of 104 enterococci per 100 milliliters for Tier (1 or 2) (daily or non-daily) use sites. Swimming areas are classified based on recreational use and are referred to as tiers.

The N.C. Recreational Water Quality Program tests water quality at ocean and sound beaches in accordance with federal and state laws. Enterococci, the bacteria group used for testing, is found in the intestines of warm-blooded animals. While it does not cause illness, scientific studies show that enterococci may indicate the presence of other disease-causing organisms. People swimming or playing in waters with bacteria levels higher than the action level have an increased risk of developing gastrointestinal illness or skin infections.

This advisory is not a beach closing, nor does the advisory affect the entire \mathbf{X} area. Swimming advisories are for waters within 200 feet of the sign. The sign posted reads as follows:

ATTENTION

SWIMMING IN THIS AREA IS NOT RECOMMENDED. BACTERIA TESTING INDICATES LEVELS OF CONTAMINATION THAT MAY BE HAZARDOUS TO YOUR HEALTH. THIS ADVISORY AFFECTS WATERS WITHIN 200' OF THIS SIGN.

OFFICE OF THE STATE HEALTH DIRECTOR

State officials will continue testing the site, and they will remove the sign and notify the public again when the bacteria levels decrease to levels below the standards.

State recreational water quality officials sample 213 sites throughout the coastal region, most of them on a weekly basis, from April to October. Testing continues on a reduced schedule during the rest of the year, when the waters are colder.

For more information on the N.C. Recreational Water Quality Program, visit the <u>program's website</u>, view a <u>map of the testing sites</u>, and follow the <u>program's Twitter feed</u>.

Website: http://www.ncdenr.gov
Facebook: http://www.facebook.com/ncdeq
Twitter: http://twitter.com/NCDEQ

RSS Feed: http://portal.ncdenr.org/web/opa/news-releases-rss 1601 Mail Service Center, Raleigh, NC 27699-1601







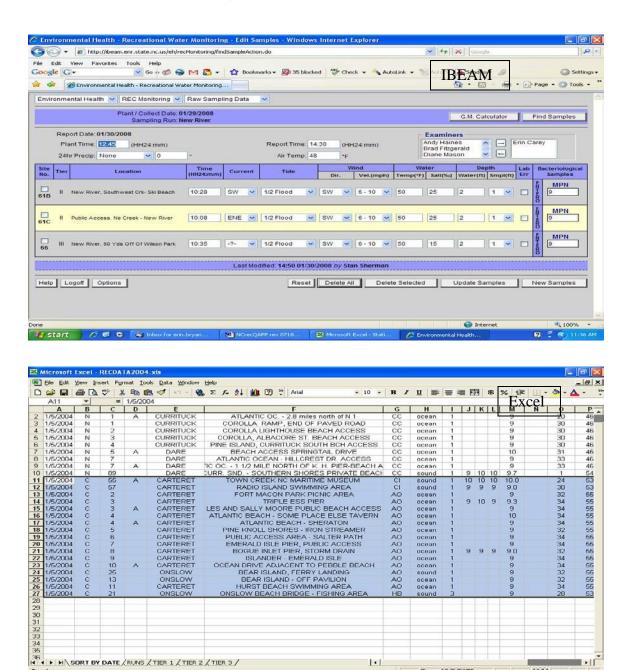
THESE WATERS MAY BE CONTAMINATED BY HUMAN OR ANIMAL WASTE. SWIMMING IS NOT ADVISED IN THESE WATERS BECAUSE OF THE INCREASED RISK OF ILLNESS.

OFFICE OF THE STATE HEALTH DIRECTOR

APPENDIX 7: Example of Field Sampling Sheet with Lab results

CDEQ	- DIVISI	NCDEQ - DIVISION OF MARINE FISHERIES - RECREATIONAL WATER QUALITY PROGRAM	ER OU	ALITY PRO	GRAM								A RESIDENT	3-404
RUN:	вос	BOGUE SOUND COUNTY: CARTERET STATE: NC	ATE:	NC		DATE COLLECTED: 4	LECTED:	14/24	3010	. U			MOHW H	SH CUI
AIR 7	TEMPE	7-2	3	J.		DATE/TIME RECEIVED IN LAB:	RECEIV	ED IN I		124/30	10	0:30	ву wном:	FRM
PREC	CIPITA'	PRECIPITATION, LAST 24 HOURS	INCHES	ES		DATE/TIME PLANTED:	PLANTI	ED: 4	18/10/30	3019		54:0	BY WHOM:	BY WHOM: IM EBM JH
E.f.	+	S.m. Media Lot#	UP 406	06		DATE/TIME REPORTED: (0/25/2019	REPOR	red: ()	125/2	019	10	10:47	BY WHOM: 1	TH
			7				WIND	S	w	WATER	DE	DEPTH	RESULTS	SSRWQ
# 12.	# 5	LOCATION OF STATION	₽ E -	TIME	Dir.	TIDE	Dir.	Vel.	Temp	% Salt	Water	Samp	Pos. Wells	MPN Entero.
22A	-	Shellrock Landing	w	20.34	SW	6	Sw	11/10	49	30	4.0	0.1	_	0
23B	0	West end of Bear Island	2	8-18	Sw	201		11/16	69	200	4.0	0 .	8	Ø17
25	W	Ferry Landing, Bear Island	w	4.8	Sw	06	Sw	11/6	49	₩ W	7.0	0.7	Q	017
27A	†	Wards Shore - b/w maker #46C & shoreline	ω (~)	62:8	SW	しのご	SW	11/16	67	ω	4.0	1,0	0	210
30	5	Ski Beach, ICWW	2	3.55	30	100	SW	11/16	49	32	5.0	01	0	017
30A	6	Bogue Inlet N of Coast Guard Channel	2	9:59	SW	106	SW	31/11	73	4	9,0	1.0	0	017
31A	7	Sound-side park at McDonald's boat ramp	3	g:05	SW	000	Sw	1//6	49	33	3.0	0.1	_	0
35A	œ	Deer Crk - Public Access end of Bogue Sd Dr	3	9:15	E	こので	SW	11/16		33	3.0	01	Ð	017
34A	۵_	Wildlife Boat Ramp in EI mouth of Archer Crk	ω (16:0	MS	207	SW	1/6	5.	34	3.0	0.1	P	210
36	0	Goose Creek, off Campground	2	96.36	S	06	SW	1/1	67	ري	5.0	1-0	-	Ø
39A	1)	Spoil Island near day marker #30	3 (9:31	SiW	200	SE	11/16	67	33	3.0	0.1	0	017
7B	()	200 yds. Off Jet Ski rental in riding area, West Salter Path	2	3:36	33	100	SW	1//1	49	83	5.0	01	6 .	017
40	3	Gales Creek, off Presbyterian Camp	2 (9:43	SW	COČ	SW	11/16	_	30	4.0	1.0	2	20
48B	エ	CCC - Aquatic Education Location	2 (9:57	(I)	LOE	SW	91/10	64	30	7.0	0	Q	017
51	15	Boat Landing Tourist Center, Morehead City	2 (9:59	UI	106	SW	11/16	67	30	12.0	1.0	Ø	410
48C	-6	Morehead City - Drain Pipe at 16th street	2	10:03	U,	100	Siv	11/16	6	33	3.0	1.0	1	0
51B	17	West end of Sugarloaf Island	2 1	0:05	C	LOG	SW	11/16	67	RE	4.0	1.0	Ø	512
47A	æ	Canal leading to Moonlite Bay	w	61:01	S	FOX	SW	41/13	6	31	G.F.	1,0	Ø.	410
47B	5	Sandbar west of Atlantic Beach high rise	2	0:15	3	70ct	3	11/16	6,1	W	رن ن	1.0	6	417

APPENDIX 7 Continued: Example of IBEAM and Excel Databases



Recreational Water Quality Program—an overview of the new standard

Does anyone check the waters on the coast to see if it's safe to swim and play in them? Yes, the Recreational Water Quality Program operated by the Shellfish Sanitation Section of the Division of Marine Fisheries monitors coastal waters on a year-round basis.

Since its inception in 1997, the program has fulfilled its purpose: "To protect the public health by monitoring the quality of North Carolina's coastal recreational waters and notifying the public when bacteriological standards for safe bodily contact are exceeded".

The program has identified 213 specific locations where the public swims and plays in coastal waters and has sorted the areas into three tiers, determined by use patterns and the resultant risks of someone becoming ill from contaminated water. Approximately 65 percent of these sites are Tier I sites, those with the most use. Examples of these areas include oceanfront beaches, summer camp swimming areas and jet-ski rental facilities. The other 35 percent of the sites are Tier II areas. These areas have less usage and are generally accessible only via watercraft. Examples of these sites include public access areas, boat ramps, and other places where smaller numbers of people use the water.

The ocean beaches and other high usage areas are sampled once per week from the beginning of April to the end of September. All stations are sampled twice per month during October, and then once per month in the winter and spring, November through March.

The program uses enterococci as the indicator organism for contamination, based on current US EPA recommendations. Enterococci are the organisms most consistently present in the intestinal tracts of animals and are most closely associated with incidents of human illness. The program uses an EPA-mandated standard of 35 enterococci per 100ml of water, based on a geometric mean (logarithmic average) using the most recent five sample results collected over the previous 30 days. It also uses a single-sample maximum of 104 enterococci per 100 ml of water to indicate that the water in question exceeds that standard. Exceeding either of these standards will resultin public notification procedures being activated.

When the standards are exceeded, local officials are notified, a press release is issued, and a sign is posted at the affected area, warning the public of the risks associated with swimming or playing in the water at this site. The program will continue to sample the area repeatedly. When the area ceases to exceed the standard, the sign is removed, local officials are notified again and another press release is issued.

Point-source contamination sources are also the sites of sign postings. Sources such as sewage treatment plant outfall pipes are permanently posted to warn swimmers not to swim or play in such areas. More problematic are pipes with stormwater discharges. These discharges are heavily contaminated but discharge only intermittently, during and after rainfall events. Currently the program posts these pipes when they begin discharging during a storm and removes the posting 24 hours after the discharge stops.

In an effort to raise awareness of the program and to solicit input from the public, several public meetings have been scheduled in the past years and are currently scheduled on a needed basis. These meetings will be held primarily in beach communities along the coast and will involve local officials, travel/tourism representatives, property owners and members of the general public. Media packages will be developed to publicize the meetings.

Erin Bryan-Millush leads the program from the Morehead City office. The recreational water quality program consists of five water samplers stationed in Nags Head (1), Morehead City (2), and Wilmington (1).

We hope to keep North Carolinian residents and visitors protected when swimming in theses coastal waters for generations to come!

THE FACTS: RECREATIONAL WATER QUALITY MONITORING IN NORTH CAROLINA



ATTENTION

SWIMMING IN THIS AREA IS NOT RECOMMENDED.

BACTERIA TESTING INDICATES LEVELS OF

CONTAMINATION THAT MAY BE HAZARDOUS TO YOUR HEALTH.

THIS ADVISORY AFFECTS WATERS WITHIN 200 FT. OF THIS SIGN.

OFFICE OF STATE HEALTH DIRECTOR

I saw this sign at the beach. Who put it there, and what does it mean?

It means testing shows that state and federal bacteria levels for swimming water quality were exceeded. Therefore, state officials recommend that you do not swim within 200 feet on either side of the sign. The main goal of the Shellfish Sanitation and Recreational Water Quality Section is to protect the public health by monitoring the quality of North Carolina's coastal recreational waters and notifying the public when bacteriological levels for safe bodily contact are exceeded.

The section started monitoring coastal recreational water quality in 1997. The coastal waters monitored include the ocean beaches, sounds, bays and estuarine rivers. Unfortunately North Carolina does not have a statewide monitoring program for inland recreational waters. The public should avoid fresh water swimming after heavy rain, especially near storm drains.

Are North Carolina's beaches safe for swimming?

Yes. North Carolina has miles of beaches with excellent water quality, and the state has an extensive monitoring program to test the waters and identify any temporary problems that might arise. The data that has been collected since the program began show that our swimming beaches have been under advisory for an average of less than 1 percent of the swimming season for each year. While the waters of North Carolina are generally very clean, it is important to monitor them continually, so the public can be informed of any localized problems.

How many stations do you monitor and how often do you monitor them?

The Recreational Water Quality staff tests 213 sites throughout the coastal area at different frequencies, depending on the time of year and use patterns of the site. Ocean beaches and other high usage areas are sampled once per week between April 1 and Sept. 30; lower usage areas are sampled twice per month. All stations are sampled twice per month during October, and then once per month in the winter, November through March. Staff members collect approximately 6,000 samples per year.

What are the recreational water quality levels?

The sections staff tests for a type of bacteria called enterococci, which are found in the intestines of warm-blooded animals such as birds, dogs, raccoons and people. Enterococci will not make you sick; however, it is often associated with other bacteria and viruses that can cause water-borne illness. The U.S. Environmental Protection Agency found that enterococcus closely correlates with incidence of human illness.

To comply with the swimming water quality levels set by the EPA and the state, water test results have to fall below a set average as well as a single-sample level. The average is the geometric mean of five weekly samples taken within a 30- day period. The geometric mean cannot exceed 35 enterococci per 100 milliliters of water. In addition, swimming advisories may be posted if a single sample exceeds the level set for it based on usage. Advisories based on single sample results are retested at the time of the posting.

What happens if the swimming water quality levels are exceeded?

If the swimming water quality level is exceeded at a site, the staff sends out a press release to inform the public and an advisory sign is posted at the swimming site. Discharges of stormwater and floodwater into the swimming area also trigger swimming advisories that last for 24 hours after the discharge has ended.

Where are the disease-causing organisms coming from?

Disease-causing organisms, or pathogens, can come from both human and animals. Stormwater runoff from agricultural and urban areas delivers pathogens from humans, livestock, wildlife and pets into recreational waters. Poorly treated wastewater from treatment plants, malfunctioning septic systems and boat discharges are sources of human fecal contamination. Bacteria can also be introduced directly into the bathing area from swimmers.

Will I get sick if I swim in waters under a swimming advisory?

Not necessarily, but you are at an increased risk.

What kind of illnesses can I contract from swimming in polluted waters?

The most common are diarrheal diseases that can be caused by bacteria, viruses and parasitic protozoa. Ear, nose, throat, skin and respiratory infections are also commonly associated with swimming in contaminated water.

What should I do if I become ill after swimming?

If you develop diarrhea or an infection after swimming in North Carolina's coastal waters, seek medical treatment and then please contact the Shellfish Sanitation and Recreational Water Quality Section of the Division of Marine Fisheries at (252) 726-6827. The Recreational Water Quality Program's staff would like to know about any possible water-borne illness outbreaks as soon as possible to prevent more people from becoming ill.

How long does a swimming advisory stay posted?

If the advisory is issued due to the single sample maximum level, it will be re-sampled daily. The sign will remain posted as long as the standard is exceeded. This means the result of the immediate resample may lift the advisory as quickly as 24 hours after posting. Once the geometric mean exceeds the

standard, the swimming advisory is not lifted until two consecutive weekly samples meet the EPA standard of 35 enterococci per 100 milliliters.

I have more questions - where can I go for answers?

If you have further questions about the Recreational Water Quality Program, you can call Erin Bryan-Millush with the Program at (252) 726-6827 or view the Programs Website at http://portal.ncdenr.org/web/mf/recreational-water-quality.

Printing of this document was funded entirely through a grant from the U.S. EPA.

STORMWATER DRAINPIPE SIGNS IN NORTH CAROLINA





What do these signs mean?

These signs are posted where stormwater pipes are discharging water into coastal swimming areas. They recommend that people do not swim within 200 feet on either side of the sign. You may see these signs at drainpipes in Carteret, Dare and New Hanover counties. The flow may not be visible if the mouth of the pipe is in the surf. If so, the sign on the top left will be posted.

People are advised not to swim in the area when the pipe has stormwater coming from it. The Recreational Water Quality Program's tests have shown that after rainfall, the runoff coming out of the pipe often exceeds state and federal standards for bacteria. Swimming in the waters near the pipe can cause an increased risk of illness.

What is stormwater runoff?

When rain falls, the water that isn't able to sink into the ground washes everything lying on hard surfaces (roads, driveways, roofs and parking lots) into pipes, some of which empty into coastal waters. The contaminants on the ground can include pet and wildlife waste, gas and petroleum products, pesticides and fertilizers. The state's Recreational Water Quality program tests for bacteria found in the intestines of warm-blooded animals, including people. If it is present in the water at high enough levels, people swimming or playing in the water run an increased risk of developing a gastrointestinal illness (diarrhea and/or vomiting) or a skin infection, particularly people with compromised immune systems. It is important to note that unlike some states, North Carolina does not have sanitary sewer outfalls discharging from pipes to our ocean beaches. Our stormwater collection systems are separate from the wastewater treatment systems and do not connect with those pipes.

Why are these signs displayed?

Past data show that stormwater tends to have high bacteria counts. Therefore, people are warned to swim away from the signs, so they don't expose themselves to an increased risk of illness. This way, if people see discharge coming from the pipe, they can play it safe and avoid swimming near it.

Is it okay for my children to play in the ponds and streams created by these pipes?

Some pipes discharge onto the beach sand, creating a pond or stream. Some parents like their children to play in these puddles or ponds because they think the children are safer away from the waves and current, but this is not a good idea. These ponds are different from natural tidal pools in that they contain all the pollutants of stormwater without the dilution effect of the ocean. Allowing children to play in them, particularly small children who may swallow water, exposes them to an increased risk of getting sick.

Will I get sick if I swim in waters under a swimming advisory? What kind of illnesses could I get from swimming in polluted waters?

Not necessarily, but you are at an increased risk. The most common illnesses are gastrointestinal diseases with symptoms such as diarrhea and vomiting. Ear, nose, throat, skin and respiratory infections are also commonly associated with swimming in contaminated water. If you become ill after swimming in North Carolina's coastal waters, seek medical treatment and then please contact us at the phone number below. We would like to know about any possible waterborne illnesses as soon as possible to prevent others from becoming ill. You may contact our office at

(252) 726-6827 or by e-mail at erin.bryan-millush@ncdenr.gov or visit us online at

http://portal.ncdenr.org/web/mf/recreational-water-quality

Printing of this document was funded entirely through a grant from the U.S. EPA.

APPENDIX 9: Laboratory Quality Assurance Plan NORTH CAROLINA

SHELLFISH SANITATION LABORATORIES

RECREATIONAL WATER QUALITY MONITORING QUALITY ASSURANCE PLAN

Effective June 2, 1997

Revised June 10, 2002

Revised May 15, 2003

Revised April 30, 2008

Revised May 5, 2009

Revised May 1, 2012

Revised Sept. 22, 2016

Revised March 15, 2017

Revised October 11, 2017

Revised April, 2021

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	mEl Agar		
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	Triphenyltetrazolium Chloride (TTC)		
	Brain Heart Infusion Broth		
	Phosphate Buffered Saline		
	Brain Heart Infusion Broth + 6.5% NaCl		
	Brain Heart Infusion Agar		
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1. INTRODUCTION

The **Quality Assurance Plan (QA plan)** is intended to add additional standardization to the Shellfish Sanitation and Recreational Water Quality laboratories and is designed to reduce errors caused by analysts, equipment, supplies and analytical methods. The full implementation of the plan will substantiate the validity of the data and produce results of high integrity.

All personnel with laboratory responsibilities will have an active role in the Quality Assurance Program with overall implementation the responsibility of the analyst-in-charge of individual laboratories.

quality assessment. Quality control consists of a set of measures intended to identify and ensure proper functioning of materials, equipment and procedures which directly impact the quality of data. Quality assessment involves evaluation of both those established procedures

The major components of the **QA plan** are **quality control** and

and the implementation of those procedures.

Performance evaluations may be internal or external and are a means of asserting and validating the level of effectiveness of the **QA plan**. Results of evaluations will be maintained in each laboratory and in the Morehead City office files. The Food and Drug Administration and the State Laboratory of Public Health conduct laboratory evaluations of the Shellfish Sanitation and Recreational Water Quality Programs. The laboratories participate in NSSP Shellfish Split Sample Proficiency testing annually and Potable Water Proficiency testing for the State Laboratory of Public Health twice a year.

2. PERSONNEL CHART

Shellfish Sanitation and Recreational Water Quality Laboratory Management Structure

Erin Bryan-Millush

Laboratory Supervisor Recreational Water Quality Program Supervisor Quality Assurance Officer

Wayne Hall

Environmental Tech. II

Amanda Hewes

Microbiological Laboratory Technician Manager of Morehead City Laboratory

Holly White

Microbiological Laboratory Technician Manager of Southern Region Laboratory

Katherine C McAvoy

Microbiological Laboratory Technician Manager of Northern Region Laboratory

Assistants

Zach Fasking

Env. Tech II Morehead City Lab

Phil Piner

Env. Tech II Morehead City Lab

Timmy Moore

Env. Specialist Morehead City Lab

Jason Hill

Env. Specialist Morehead City Lab

Kenny Midgett

Env. Specialist N. Region Lab

Matthew Stokley

Env. Tech II S. Region Lab

Lucas Edmonson

Env. Specialist S. Region Lab

3. TRAINING REQUIREMENTS

The laboratory supervisor will be responsible for ensuring that all staff are adequately trained to perform the procedures assigned. Training will be provided by the laboratory Quality Assurance Officer. Trainees will be required to demonstrate proficiency prior to completion of training.

All personnel working in the laboratory will be trained in the following areas:

- preparation of media and reagents
- calibration of instrumentation
- sample preparation and inoculation
- laboratory safety
- quality control

4. GENERAL LABORATORY PRACTICES

The procedures and protocols of analyses are defined in <u>Laboratory Procedures for the Examination of Sea Water and Shellfish</u>, 4th Edition; <u>Bacteriological Analytical Manual</u>, 8th Edition, 1995; EnterolertTM-A Rapid Method for the Detection of Enterococcus spp., Idexx Laboratories, Inc., Westbrook, ME; <u>Method 1600: Enterococci in Water by Membrane Filtration Using membrane-Enterococcus Indoxyl-β-D-Glucoside Agar (mEI) and <u>Standard Methods for the Examination of Water and Wastewater</u>, 20th Edition, 1998. General guidelines for laboratory practices are listed below.</u>

- a. Proper labeling, storage and/or disposal of supplies.
- b. Laboratory:
 - bench-tops will be disinfected before and after each use
 - work area adequate for workload and storage
 - work area clean and well lighted.
- c. Environmental contamination beyond laboratory control will be reported promptly to the Quality Assurance Officer and/or the Laboratory Supervisor.
- d. The laboratory staff is responsible for reporting equipment breakdowns to the Laboratory Supervisor.
- e. Laboratory staff will keep the laboratory prepared to handle all regularly schedule workloads at all times.
- f. A copy of the **Quality Assurance Plan** will be located in an accessible area for all personnel to obtain.
- g. Records are maintained in the Shellfish Sanitation and Recreational Water Quality Section quality assurance notebook.

5. QA - MATERIALS

5.1 Glassware

- a. All broken glassware will be discarded.
- b. Glassware will be of borosilicate non-fluorescent glass or other non-toxic material.
- c. Culture tubes and durham tubes will be non-fluorescent.
- d. Washing and sanitizing will be in accordance with procedures in <u>Laboratory</u> <u>Procedures for the Examination of Seawater and Shellfish, 4th Edition</u>.
- e. Inhibitory residue test will be performed after glassware is washed and dried using Bromothymol Blue solution, but only if detergents are used for washing glassware.

5.2 Pipets

- a. Will be borosilicate glass or other non-toxic disposable plastic.
- b. Pipets conform to the requirements cited in <u>Recommended Procedures for the Examination of Sea Water and Shellfish</u>.
- c. Sterility of reusable pipets will be determined after each sterilization.
- d. Efficiency of hot air drying oven will be tested with spore strips quarterly to ensure proper sterilization of pipettes.
- e. Sterility of disposable pipets will be determined for each batch.

5.3 Dilution Bottles

- a. Will be borosilicate glass or other non-toxic material.
- b. Graduation levels marked on side.
- c. Screw caps used with glass sample vessels. Use autoclave tape on the cap to show the sample vessels are sterile and also for labeling sample number.

5.4 Sample Bottles

- a. Will be borosilicate glass or suitable non-toxic material capable of being washed and sterilized.
- b. Will contain sufficient volume of sample for all required test plus adequate space to allow for effective shaking.

c. Will be checked for sterility after every sterilization.

5.5 Inoculating Equipment

- a. Wire loops may be used as recommended in <u>Recommended Procedures for the Examination of Seawater and Shellfish.</u>
- b. Single service disposable hardwood applicators may be used and will be 0.2 to 0.3 cm in diameter and at least 2.5 cm longer than fermentation tubes; sterilized in autoclave, dried in hot air oven and stored in stainless steel containers. Sterility of hardwood applicator sticks will be tested after each sterilization. See data sheet on page 27.
- c. Sterile disposable inoculating loops, 10 µl or 1µl may be used.

5.6 Dehydrated Media and Enterolerttm Reagent Snap Packs

- a. Stored as specified on the manufacturer's label.
- b. All media received is labeled with date received and date opened.
- c. Lot number and expiration date will be recorded for each batch of media.
- d. Dehydrated media which is discolored, caked, or expired is properly disposed (or sent back to central office for manufacture reimbursement).
- e. Enterolert reagent snap packs are discarded when expired or sent back to central office for manufacture reimbursement if the snap pack appears to be damaged and/or punctured and the contents appear to be discolored or caked.

5.7 Water

- a. Only deionized or distilled water is used for media and reagent preparation.
- Deionized water supply is monitored for conductivity using the Myron 750 Series II
 Digital Conductivity Controller to ensure that conductivity is less than 2 micro
 Siemens.
- c. Make up water will be tested monthly for pH (5.5 7.5), total residual chlorine (mg/L < 0.1) and bacterial level (<1000 CFU/mL) and annually for dissolved metals (Cd, Cr, Cu, Ni, Pb, Zn).
- d. Stored volumes of sterile, lab pure water are replaced frequently.

5.8 Media Preparation

a. Culture media preparation is according to the method of analysis and will be as

described in <u>Laboratory Procedures for the Examination of Seawater and Shellfish, 5th Edition</u> and <u>Standard Methods for the Examination of Water and Wastewater, 20th Edition.</u>

- b. Membrane Filtration culture media preparation is according to <u>Method 1600:</u> Enterococci in <u>Water by Membrane</u> Filtration <u>Using membrane-Enterococcus Indoxyl-β-D-Glucoside Agar, (mEI)</u>
- c. SPC agar in flasks and Nutrient broth will be stored under refrigeration for a period not to exceed three months.
- d. All prepared media will be properly labeled with type and preparation date.

6. UNBUFFERED DILUTION WATER

- a. Deionized water is to be sterilized for 12 minutes at 121°C and stored at room temperature.
- b. Place deionized water in 500 mL flask with screw cap. Fill to below 450 mL graduation. Mark cap with sterilization tape and back off 1/4 turn.
- c. For storage of all deionized water, rotate flasks so that most recently prepared water will be used last. Write sterilization date on sterilization tape on cap.

7. MEDIA PREPARATION

7.1 ENTEROLERT™ used to enumerate Enterococci

One snap pack of reagent for 100 mL water sample. Order from IDEXX Laboratories, Inc., One IDEXX Drive, Westbrook, Maine 04092 (1-800-321-0207).

7.2 mEl Agar – used to enumerate Enterococci

Combine ingredients and heat to boil for 1 minute in 4 L stainless steel beaker. Stir continuously to prevent burning and sticking of media. When completely dissolved and tempered in waterbath check pH, record and adjust by adding 1 mL of 1M Sodium Hydroxide at a time until range is met. Pour hot media equally into two 1 L flasks, to prevent boiling over in autoclave. Sterilize in autoclave at 121°C for 12 minutes. Remove from autoclave when chamber pressure reaches "0". Check pH, and record (range between 7.1 \pm 0.2). Temper in waterbath. Once tempered add measured amount of Nalidixic Acid and Triphenyltetrazolium Chloride. Dispense 5 mL of warm agar into 9x50mm petri dishes and allow to solidify. Store upside down in a \leq 4°C refrigerator.

7.3 Nalidixic Acid – added to mEl agar to enumerate Enterococci

Ingredients:	
Nalidixic Acid – 0.48 g	
10 N NaOH – 0.4 mL	
Deionized	water – 10 ml

Combine ingredients and filter-sterilize. Add to tempered mEI agar at 5.2 mL per liter.

7.4 Triphenyltetrazolium Chloride (TTC) - added to mEI agar to enumerate Enterococci

Ingredients:
Triphenyltetrazolium Chloride (TTC) – 0.1 g
Deionizedwater – 10 mL

Combine ingredients and filter-sterilize. Add to tempered mEI agar at 2 mL per liter.

7.5 Brain Heart Infusion Broth (BHIB) - used in the colony verification process

Ingredients:

Commercially prepared DIFCO™ Brain Heart Infusion Broth (BHIB) – 37 g

Deionized water – 1 L

Combine ingredients in 4 L stainless steel beaker. Stir and allow media to go into solution. When completely dissolved, check pH, record, and adjust if necessary by adding 1 mL of 1M Sodium Hydroxide at a time until range is met. Dispense 9-10 mL of media into 150 mm X 20 mm screw cap culture tubes. Caps backed off 1/4 turn, label and date. Sterilize in autoclave at 121°C for 12 minutes. Remove from autoclave when chamber pressure reaches "0". Check pH, and record (range between 7.4 ± 0.2). Cool at room temperature. Tighten caps and refrigerate.

7.6 Brain Heart Infusion Broth (BHIB) with 6.5% NaCI - used in the colony verification process

Combine ingredients in 4 L stainless steel beaker. Stir and allow media to go into solution. When completely dissolved, check pH, record, and adjust if necessary by adding 1 mL of 1M Sodium Hydroxide at a time until range is met. Dispense 9-10 mL of media into 150 mm X 20 mm screw cap culture tubes. Caps backed off 1/4 turn, label and date. Sterilize in autoclave at 121°C for 12 minutes. Remove from autoclave when chamber pressure reaches "0". Check pH, and record (range between 7.4 ± 0.2). Cool at room temperature. Tighten caps and refrigerate.

7.7 Brain Heart Infusion Agar (BHIA) - used in the colony verification process

Combine ingredients and heat to boil for 1 minute in 4 L stainless steel beaker. Stir continuously to prevent burning and sticking of media. When completely dissolved and tempered in waterbath check pH, record and adjust by adding 1 mL of 1M Sodium Hydroxide at a time until range is met. Dispense 9-10 mL of hot media into 150 mm X 20 mm screw cap culture tubes. Caps backed off 1/4 turn, label and date. Sterilize in autoclave at 121° C for 12 minutes. Remove from autoclave when chamber pressure reaches "0". Check pH, and record (range between 7.4 ± 0.2). Cool at room temperature with culture tubes at an angle, so agar solidifies as a slant. Tighten caps and refrigerate.

7.8 Bile Esculin Agar (BEA) - used in the colony verification process

Combine ingredients and heat to boil for 1 minute in 4 L stainless steel beaker. Stir continuously to prevent burning and sticking of media. When completely dissolved and tempered in waterbath check pH, record and adjust by adding 1 mL of 1M Sodium Hydroxide at a time until range is met. Dispense 9-10 mL of hot media into 150 mm X 20 mm screw cap culture tubes. Caps backed off 1/4 turn, label and date. Sterilize in autoclave at 121° C for 12 minutes. Remove from autoclave when chamber pressure reaches "0". Check pH, and record (range between 6.6 ± 0.2). Cool at room temperature with culture tubes at an angle, so agar solidifies as a slant. Tighten caps and refrigerate.

7.9 Nutrient Broth – used for routine QA check of sample collection bottles and pipettes

Ingredients: Commercially prepared DIFCO TM Nutrient Broth -8~g Deionized water -1~L

Combine ingredients in 4 L stainless steel beaker. Stir and allow media to go into solution. When completely dissolved, check pH, record, and adjust if necessary. Dispense media into 150 mm X 20 mm screw cap culture tubes. Caps backed off 1/4 turn, label and date. Sterilize in autoclave at 121° C for 12 to 15 minutes. Remove from autoclave when chamber pressure reaches "0". Check pH, and record (range between 6.8 ± 0.2). Cool at room temperature. Tighten caps and refrigerate.

7.10 Standard Methods Plate Count Agar - used for routine QA check of DI system

Ingredients:

Commercially prepared DIFCO™ Plate Count Agar – 23.5 g Deionized water – 1 L

Combine ingredients in 4 L stainless steel beaker. Stir to mix thoroughly. Heat to boil (using double boiler). Boil for 1 minute to completely dissolve media. When completely dissolved and tempered in waterbath check pH, record and adjust by adding 1 mL of 1M Sodium Hydroxide at a time until range is met. Dispense media into 150 mm X 20 mm screw cap culture tubes. Caps backed off 1/4 turn, label and date. Sterilize in autoclave at 121° C for 12 to 15 minutes. Remove from autoclave when chamber pressure reaches "0". Check pH, and record (range between 7.0 \pm 0.2). Cool at room temperature. Tighten caps and refrigerate.

8. QA - EQUIPMENT MAINTENANCE AND MONITORING

8.1 Thermometers

- a. Will be clearly marked and readable.
- b. Calibrated against NIST standards thermometer annually; labeled and dated at last calibration with ± temperature fluctuation.
- c. NIST standards thermometer calibrated annually by a certified laboratory.
- d. Ice point of NIST standards thermometer to be determined annually by certified laboratory or by section laboratory staff.
- e. Will be properly immersed as required by manufacturer.
- f. Air incubator thermometers will be immersed in liquid.
- g. Graduated at 0.1°C intervals for incubators and water baths.

8.2 Air Incubators

- a. Shall maintain a uniform and constant temperature and must not vary more than \pm 0.5°C in the areas used.
- b. Will be of adequate capacity for work load.
- c. Twice daily temperature records maintained by analyst with at least 4 hours between measurements.

8.3 Water Bath

- a. Shall maintain temperature of 45.0 ± 0.2 °C.
- b. Twice daily temperature records maintained by analyst with at least 4 hours between measurements.
- c. To be covered, and adequate water level maintained.
- d. Water mechanically circulated.
- e. Cleaned and disinfected quarterly (more often if needed).

8.4 Autoclave

- a. Will maintain a pressure range of 15 to 18 psi. during sterilization.
- b. Temperature maintained between 120-123°C for sterilization. Maximum registering

- working thermometer to be used with every load and records maintained. Working thermometer to be checked against a NIST calibrated maximum registering thermometer yearly.
- c. Routine cleaning weekly to include drain trap.
- d. Spore suspensions (*Bacillus stearothermophilus*) will be used monthly to evaluate the effectiveness of the autoclave sterilization process and results recorded. Heat sensitive tape will be used with each autoclave use.
- e. NIST calibrated maximum registering autoclave thermometer to be re-calibrated every 5 years.
- f. Autoclaves will be serviced annually, or as needed, by a qualified technician and records maintained.
- g. Autoclave cycle to be timed quarterly.

8.5 Balances

- a. Provide a sensitivity of 0.1 g at a load of 150 g.
- b. Calibrated monthly using NIST class S weights.
- c. Calibration records maintained.

8.6 pH Meter

- a. Have standard accuracy of 0.1 pH unit.
- b. Calibrated with 2 buffers that include correct pH range of medium being prepared. Choosing between 3 standard buffers of 4.0, 7.0 and 10.0. Standard buffer solutions are used fresh daily.
- c. Electrode efficiency determined with each use (record slope after calibration).
- d. Calibration log to be maintained.

8.7 Hot Air Drying Oven

- a. Temperature for sterilization of glassware will be maintained at 170° C.
- b. Spore strips (*Bacillus subtilis*) will be used quarterly to evaluate efficiency of sterilization.

9. QUALITY CONTROL CHART

APPARATUS	TEST	FREQUENCY
Thermometers	NIST Calibration	Annually
Water System	Conductivity	Monthly
	Chlorine	Monthly
	pH	Monthly
	Standard Plate Count	Monthly
	Heavy Metals	Annually
Autoclave	Maximum registering temp.	Each use
	B. stearothermophilus	Monthly
	Time Cycle	Quarterly
Balance	Calibration –	Monthly
	NIST Class S weights	,
pH Meter	Calibration – 2 out of 3 buffers	Each use
	Electrode efficiency	Each use
Incubators	Record temperature	Twice daily
Waterbaths		
Refrigerators		
Drying Oven	Record temperature	Each use
	Spore strips	Quarterly
Glassware	Pipette sterility	Each sterilization and Monthly
		in use
	Bottle sterility	Each sterilization and Monthly
		in use
Plastic ware	Disposable pipette sterility	Each batch
	IDEXX vessel sterility	Each lot number
Sterilized deionized water	Water sterility	Each sterilization

10. ANALYSES PERFORMED

PROCEDURES FOR THE ENUMERATION OF ENTEROCOCCUS SP. IN SEAWATER

10.1 <u>Enterococcus sp.</u> detection using EnterolertTM reagent fluorescence method.

- a. Pour sterile deionized water into a sterile, transparent, non-fluorescent, borosilicate glass vessel to the 90 mL fill line. Repeat for the number of samples to be processed. Also fill three additional vessels to the 100 mL fill line for quality control.
- b. Label the autoclave tape on top of the sterilized sample vessels in consecutive numerical order and the quality control vessels as media, E.f. (*Enterococcus faecium* positive control), and S.m. (*Serratia marcescens* negative control). Inoculate the controls.
- c. Number and date the 51-well Quanti-Trays[™] on the paper side in the same consecutive order as the sample vessels. Label the control trays as above and include the date.
- d. Count number of Enterolerttm snap packs to be used. Carefully separate the snap packs.
- e. Up to 30 minutes prior to the receipt of samples in the lab, add the contents of one Enterolerttm snap pack to each glass sample vessel containing 90 mL sterile deionized water. Shake to completely dissolve reagent. Un-dissolved reagent particles may cause green fluorescence in Quanti-Tray wells after incubation. Hold at room temperature.
- f. Turn on the Quanti-Tray™ Sealer at least 10 minutes prior to use.
- g. Seawater samples kept on ice or refrigerated at or below 10°C before examination. Temperature control collected in field by samplers and examined when received in laboratory. Samples will be processed within 6 hours of sampling.
- h. Samples are to be shaken 25 times within 7 seconds in a 1 foot arc.
- i. 10 mL from water sample bottle is pipetted into a glass sample vessel containing 90 mL sterile deionized water and Enterolert media, making the dilution for marine waters. Cap and mix well before transferring to the Quanti-Tray. Repeat procedure for all samples and controls.
- j. After reagent has dissolved, pour sample into Quanti-Tray[™] following Enumeration Test Procedure 1 through 4 from IDEXX brochure.
- k. Place sample filled Quanti-TrayTM onto rubber tray carrier with plastic well side down. Insert carrier and tray into Quanti-TrayTM Sealer and remove from Sealer once the tray is sealed. Repeat for all samples and controls.
- I. Incubate at 41°± 0.5° for 24 hours. Do not stack trays more than 5 high and leave space between stacks in the incubator. It is critical to maintain correct and stable temperature throughout the entire incubation time to prevent false positives from occurring in Enterolert.
- m. During incubation, if certain samples are suspected to yield high counts, samples can be removed briefly at the 20-hour mark for examination of positive fluorescent wells, at least 95% of growth will have taken place (Gil Dicter, gil-dicter@idexx.com). Samples must be immediately returned into the incubator for the remaining incubation period. It is important to keep this practice to a minimum to reduce the risk for false positive or false negative results. "This indicator bacterium will exponentially double every 20 minutes (bacterial growth curve)

and if present even at levels as low as 2/100ml will fluoresce under 365 nm UV light in less than 24 hours and fluorescence can be observed at 20 hours. Trays can be observed for the presence or absence of fluorescence prior to 24 hours and then placed back into the incubator for total time of 24 hours and should have no detrimental effect on the final results. If all the wells fluoresce at that time further incubation is not required." By Gil Dicter, IDEXX Labortories.

- n. Final examination of Quanti-Trays should be conducted at the 24 hour time period. Under incubation (read too early) would cause positive wells to be too weak to read. Over incubating (read too late) would increase the chances of false positives.
- o. Look for fluorescence with a 6-watt, 365-nm, UV light within 5 inches of the sample in a dark environment. Face the light away from your eyes and towards the sample.
- p. When reading results from marine water or brackish water samples, it is not recommended to leave the samples on the bench exposed to light or under the UV lamp too long before collecting results.
- q. Count positive (fluorescent) wells and refer to the 51-well Quanti-Tray[™] MPN table for results. The MPN value is multiplied by a factor of 10 due to the 1/10 dilution made for marine waters.

Result Interpretation

Lack of fluorescence = negative for entercocci Blue fluorescence = positive for enterococci

- 10.2 <u>Enterococcus sp.</u> detection using <u>Method 1600: Enterococci in Water by</u>
 <u>Membrane Filtration Using membrane-Enterococcus Indoxyl-β-D-Glucoside</u>
 <u>Agar</u>, (mEI)
 - a. Mark petri dishes containing mEI Agar with sample identification and sample volume on the back-side of the dish containing agar.
 - b. Place a sterile membrane filter on the filter base, grid-side up and attach the funnel to the base so that the membrane filter in now held between the funnel and the base. *NOTE: When using a new funnel, the same funnel may be used for multiple volumes for the same site starting with the smallest volume first.

- c. Shake sample bottle 25 times to distribute the bacteria uniformly, and measure the desired volume of sample into the funnel, starting with the smallest volume first. Leave pipette in sample bottle for distributing of additional volumes. It is not necessary to shake sample between concurrent sample volumes, use pipette to stir sample by drawing up and down with pipette aid twice before measuring sample volume. Current volumes as of November 2016 are 30mL, 60mL and 80 mL. *NOTE: When analyzing smaller volumes (e.g., <20mL), add 20-30 mL of PBS to the funnel. This will allow even distribution of the sample on the membrane.
- d. Filter the sample, and rinse the sides of the funnel at least twice with 20-30 mL of sterile buffered rinse water. Turn off the vacuum and remove the funnel from the filter base.
- e. Use sterile forceps to aseptically remove the membrane filter from the filter base, and roll it onto the mEI agar to avoid the formations of bubbles between the membrane and the agar surface. Reset the membrane if bubbles occur. Run the forceps around the edge of the filter outside the area of filtration, close to the edge of the dish, to be sure that the filter is properly seated on the agar. Close the dish, invert, and place into a tight fitting plastic container.
- f. Process controls to accompany samples throughout incubation.
 - I. Media sterility: place one plate of sterile mEI agar into plastic container
 - II. Method blank: measure 50 mL of sterile PBS and follow steps d. and e. *NOTE: you can use the same filter funnel used for the Method blank for the Negative control.
 - III. Negative control: inoculate 100 mL of PBS with *E. coli* from dilute suspension. Shake to mix and follow steps d. and e., dispose funnel.
 - IV. Positive control: inoculate 100 mL of PBS with 1 BioBall™ containing *E. faecalis*. Shake vigorously 25 times to dissolve, allow contents to settle for ~5 minutes then using a new filter funnel, pour contents in filter funnel *NOTE: you may need to rinse inside of dilution bottle and pour additional contents into filter funnel to ensure all cells are filtered, then follow steps d. and e.
 - V. When using the UV sterilizer, be sure to process an extra method blank for every 15 samples processed.
- g. Place plastic container with inverted plates in air Incubator at $41^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ for 24 ± 2 hours. Record time placed in air incubator on sampling sheet.
- h. Using refractometer, read salinity of each sample and record on sampling sheet.
- i. Record sterilization date of mEI agar used for analysis on sampling sheet.
- j. After incubation count and record colonies on those membrane filters containing the acceptable range of 20-60 colonies ≥0.5 mm in diameter with a blue halo regardless of colony color. Refer to Method 1600 EPA-821-R-09-016 appendix B: C.3.6
 - I. If all membrane filters are below the acceptable range of 20-60 colonies, select the most nearly acceptable count or counts (if two are more counts are equal but have different volumes than take the average of those numbers as the final CFU and record result on sampling sheet.
 - II. If counts from all membrane are zero, calculate using count from largest filtration volume. Calculate the number of colonies per 100 mL that would have been reported if there was one colony on the filter representing the largest filtration volume. Report this result as a less than value.

k. Results are reported as CFU/100 mL of sample.

Result Interpretation

Colonies with a blue halo regardless of colony color = positive for enterococci

Verification Procedure

Colonies ≥0.5 mm in diameter of any color having a blue halo after incubation on mEI agar are considered to be "typical" enterococci colonies. Verification of colonies may be required in evidence gathering and it is also recommended as a means of quality control. The verification procedure follows.

- a. Using a sterile inoculating loop or needle, transfer growth from the centers of at least 10 well-isolated typical and at least 10 well-isolated atypical colonies into a BHIB tube and onto a BHIA slant. Incubate broth for 24 ± 2 hours and agar slants for 48 ± 3 hours at 35° C $\pm 0.5^{\circ}$ C.
- b. After a 24-hour incubation, transfer a loopful of growth from each BHIB tube to BEA, BHIB, and BHIB with 6.5% NaCl.
 - I. Incubate BEA and BHIB with 6.5% NaCl at 35° C $\pm 0.5^{\circ}$ C for 48 ± 3 hours.
 - II. Incubate BHIB at 45° C $\pm 0.5^{\circ}$ C for 48 ± 3 hours.
- c. Observe all verification media for growth.
- d. After 48-hour incubation, perform a Gram stain using growth from each BHIA slant.
- e. Gram-positive cocci that grow and hydrolyze esculin on BEA (i.e., produce a black or brown precipitate), and grow in BHIB with 6.5% NaCl at 35°C ± 0.5°C and BHIB at 45°C ± 0.5°C are verified as enterococci.

11.LAB FORMS -

- (1) Reference Master Lab Sheets on shared drive for the current up-to-date documents used to verify quality control and quality assurance on items used to process recreational water quality samples.
 - a. LBS 1 Temperature Records ALL
 - b. LBS 2 Bromothymol Blue Test
 - c. LBS 5 Autoclave Sterilization Record
 - d. LBS 8 Oven Drying Pipettes